MUSEUM LIBRARY
THE UN... OF MICHIGAN
2 1962
MUSEUMS LIBRARY

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XV *December 1961* No. 4

CONTENTS

Population Dynamics of a Variant *t*-Allele in a Confined Population of Wild House Mice. L. C. Dunn and Howard Levene ... 385
Divergence in *Tripsacum* and *Zea* Chromosomes. Marjorie P. Maguire ... 394
Courtship and Sexual Isolation in *Drosophila pavani* Brncic and *Drosophila gaucha* Jaeger and Salzano. Susi Koref-Santibañez and Eduardo del Solar O. ... 401
Galápagos Tomatoes and Tortoises. Charles M. Rick and Robert I. Bowman ... 407
Hybrid Swarms Between Wild and Cultivated Rice Species, *Oryza perennis* and *O. sativa*. Hiko-Ichi Oka and Wen-Tsai Chang ... 418
Historical Zoogeography of Australian Mammals. George Gaylord Simpson ... 431
Frequencies and Allelism of Lethal Factors Within and Between Gene Arrangements. I. San Jacinto Mountains. Carl Epling, Victor E. Tinderholt, and R. H. T. Mattoni ... 447
Floral Evolution in the Family Gramineae. H. T. Clifford ... 455
The Evolutionary Status of *Drosophila serrata*. Theodosius Dobzhansky and Wharton B. Mather ... 461
Evolution in a Group of Indo-Australian *Rasenes* (Hymenoptera, Eumenidae). J. van der Vecht ... 468
Food-Plant Specialization in the Moth *Panaxia dominula* L. L. M. Cook ... 478
Peculiar Gene Arrangements in Brazilian Natural Populations of *Drosophila ananassae*. N. Freire-Maia ... 486
Heterosis and Fitness in Experimental Populations of *Drosophila melanogaster*. Hampton L. Carson ... 496
Outlines of Evolution in the Pseudogamous *Ranunculus auricomus* Group in Finland. Gunnar Mærklund and Arne Rousi ... 510
Studies on European Lampreys. Giuseppe S. J. Zanandrea ... 523
Chromosomal Adaptive Polymorphism in *Drosophila persimilis*. III. Mating Propensity of Homokaryotypes. Eliot B. Spiess and Bozena Langer. Appendix by C. C. Li ... 535
The East Pacific Barrier and the Distribution of Marine Shore Fishes. John C. Briggs ... 545
Tests for Social Discrimination Between Three Species of the *Peromyscus truei* Species Group of White-footed Mice. J. R. Tamsitt ... 555
Notes and Comments
A Possible Phylogenetic Interpretation of the Annelidicolous Copepod *Phyllocola petiti* Delamare & Laubier. R. V. Gotto ... 564
Larval Competition and the Origin of Tetrapods. Frederick E. Warburton and Norris S. Denman ... 566
Studies in Warning Coloration and Mimicry. III. Evolution of Müllerian Mimicry. James E. Huheey ... 567
Books Received ... 568
Report on the Sixteenth Annual Business Meeting of the Society for the Study of Evolution ... 569

PUBLISHED QUARTERLY

BY THE SOCIETY FOR THE STUDY OF EVOLUTION

1941 NEW HAMPSHIRE STREET, LAWRENCE, KANSAS

ISSUED DECEMBER 30, 1961

EDITORIAL BOARD

I. Michael Lerner, *Editor*

D. Dwight Davis, *Managing Editor*

ASSOCIATE EDITORS

Class of 1961

R. B. Cowles, *Los Angeles*
A. B. da Cunha, *São Paulo*
R. C. Rollins, *Cambridge (Mass.)*
D. B. O. Savile, *Ottawa*
R. A. Stirton, *Berkeley*
P. V. Terentjev, *Leningrad*

Class of 1962

D. G. Catcheside, *Birmingham*
V. Grant, *Claremont*
R. C. Lewontin, *Rochester*
S. Makino, *Sapporo*
J. N. Spuhler, *Ann Arbor*
M. Westergaard, *Copenhagen*

Class of 1963

W. F. Blair, *Austin*
L. L. Cavalli-Sforza, *Parma*
P. Dansereau, *New York*
G. F. Gause, *Moscow*
H. Laven, *Mainz*
C. D. Michener, *Lawrence*

EVOLUTION is published quarterly by the Society for the Study of Evolution. Membership is $7.00, including subscription to EVOLUTION. Subscription price to non-members: $10.00.

EVOLUTION is open to papers of evolutionary interest. Manuscripts and all editorial correspondence should be addressed to the Editor at the Department of Entomology, University of Kansas, Lawrence, Kansas. Subscriptions are to be ordered through the office of the Treasurer, Dr. Robert E. Beer, Department of Entomology, University of Kansas, Lawrence, Kansas.

Numbers lost in the mail will be supplied free if written claim is received within one month of the date of issue of the next succeeding number.

Notices of change of address should be sent to the Treasurer. Directions for the mailing of reprints should be addressed to EVOLUTION, 1041 New Hampshire Street, Lawrence, Kansas.

Second class postage paid at Lawrence, Kansas, U.S.A.

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. XV DECEMBER, 1961 No. 4

POPULATION DYNAMICS OF A VARIANT *t*-ALLELE IN A CONFINED POPULATION OF WILD HOUSE MICE

L. C. DUNN[1] AND HOWARD LEVENE[2]

Departments of Zoology and Mathematical Statistics, Columbia University

Received September 1, 1960

Variant alleles at one locus, *T*, have been found in many populations of wild house mice (Dunn and Suckling, 1956; Dunn, 1957b), but reliable estimates of gene frequency have not been obtained since most of the population samples tested have been small. One such population, breeding in confinement, has now been tested over a period of 7½ years, and a reliable estimate obtained of the frequency in it of one *t*-allele for which it is polymorphic. Since the allele is favored by high transmission ratio from male heterozygotes, and is subject to strong negative selection because of the sterility of males homozygous for it, the results are of interest in the study of evolutionary forces which influence the equilibrium frequencies of such genes (Dunn, 1957a).

The wild population tested is that maintained since 1944 by Dr. Howard A. Schneider at the Rockefeller Institute, New York City. The origin and maintenance of this population have been described by Dr. Schneider (1946), to whom we are indebted for generous samples of mice from it supplied to us at intervals since 1952. It originated in a composite sample of wild mice captured in New York City and Long Island in 1943, to which were added in 1944 a few wild mice captured in Philadelphia, Pa. It has been maintained without introduction of further wild mice since 1944.

Formal breeding records of the population were not kept, the object being to maintain a small population (75 to 125 individuals 1944–1950; 50 to 60 from 1951 to the time of the last sample in 1959) with avoidance of close inbreeding in an effort to maintain heterogeneity. It is likely that the population was reduced at least once to very small size, perhaps to an effective population number of only four individuals. Since 1951 only two generations per year have been bred.

RESULTS OF SAMPLING

When first tested, in January 1952, five out of a sample of eight males from this population were found to be heterozygous for a *t*-allele (Dunn and Morgan, 1953). Diagnosis of heterozygosity in this, as in subsequent tests, is based on production of tailless offspring (*T/t*) when normal wild mice are tested by mice of laboratory stock

[1] Breeding experiments carried out at Nevis Biological Station, Irvington-on-Hudson, New York, under contract AT (30-1)-1804 with U. S. Atomic Energy Commission. The assistance of Susan Goldhor and Andrew B. Beasley is gratefully acknowledged.

[2] Research sponsored by the Office of Naval Research under Contract No. Nonr-266(33), Project No. NR 042-034.

carrying a dominant marker, Brachyury $T/+$, at this locus. From one such male of this sample a tailless line T/t^{w1} was extracted which produced only tailless offspring. The t-allele in this balanced lethal line is lethal late in embryogeny (Bennett, Dunn and Badenhausen, 1959). From another male from this sample was extracted a second balanced tailless line, T/t^{w2}. Homozygotes t^{w2}/t^{w2} were viable with normal tails, females being fertile and males sterile (8 tested). The lethal allele t^{w1} was transmitted by heterozygous males T/t^{w1} to about 90% of their progeny; t^{w2} from T/t^{w2} males to about 95% of progeny. Thus the population when first tested contained two different t-alleles.

A second sample was tested in August 1952. Six animals produced tailless offspring out of 15 which yielded at least one litter, but the allele frequency was not determined since those which failed to produce tailless offspring were not exhaustively tested. Again two different t-alleles, a lethal and a viable, were shown to be present.

A third sample was tested three years later, in June 1955 (Levine and Dunn, 1956). Fifty-eight out of 99 mice which produced at least one litter produced tailless offspring. Twenty-six males and 13 females gave no litters at all after four months of mating with fertile Brachy mates. Tailless descendants of 13 tested parents proved to carry t^{w2}. No t^{w1} allele was found, although the sample size was inadequate to prove its absence from the population. It was evident that at least one allele was still present in the population in high frequency. The original data, including results of a few tests completed after the results of this test had been published, are shown in table 1. Gene frequency estimates derived from these tests will be presented below.

In 1959 another large sample from this population was tested. The results are given in table 1. Before estimating the allele frequency it is necessary to know whether more than one kind of t-allele was present. A sample of the tailless offspring from the test crosses was tested by mating with T/t^{w1}

TABLE 1. *Results of progeny tests of members of a confined population by Brachy (T/+) mice. Number of wild type ($t^w/+$ and $+/+$) not given. Br = Brachy, o^1 = Tailless (T/t^w), n = number of progenies of the given type*

1955♂			1955♀			1959♂			1959♀		
n	Br	o^1	n	Br	o^1	n	Br	o^1	n	Br	o^1
1	0	1	1	0	1	1	0	2	2	0	1
1	0	2	2	0	2	1	0	3	1	0	2
4	0	3	2	0	3	1	0	5	2	0	3
2	0	4	7	0	4	4	0	6	1	0	5
5	0	5	3	0	5	2	0	7	3	2	1
1	0	7	2	2	1	1	0	15	3	3	1
1	4	1	2	9	1	1	1	2	1	4	1
1	1	2	4	1	2	1	1	4	1	2	2
3	1	4	3	2	2	2	2	4	2	3	2
1	2	6	3	4	2	1	3	5	1	5	2
1	2	9	1	5	2	1	3	6	3	2	3
1	6	9	1	8	2	1	1	7	2	1	4
2	1	0	2	1	3	1	2	7	1	1	5
1	4	0	1	3	3	1	5	7	1	1	0
2	6	0	1	5	3	1	1	8	2	2	0
2	8	0	1	2	4	1	1	9	4	3	0
2	9	0	1	4	4	1	1	10	2	4	0
2	10	0	1	4	5	1	4	12	3	5	0
1	13	0	1	1	0	1	2	0	2	6	0
1	14	0	3	2	0	1	3	0	3	7	0
1	16	0	3	3	0	1	4	0	1	8	0
2	17	0	1	4	0	1	5	0	3	9	0
1	18	0	2	5	0	1	7	0	2	13	0
2	20	0	1	6	0	2	8	0			
1	23	0	1	7	0	2	9	0			
1	24	0	2	9	0	2	10	0			
			2	10	0	4	11	0			
			1	12	0	2	13	0			
			1	14	0	2	17	0			
			1	16	0						

tailless. One tailless offspring each from 9 different $+/t$ males and one each from 10 different $+/t$ or t/t females gave when so tested both tailless and normal-tailed offspring. The latter are interpreted as t^{w1}/t^{ws}, t^{ws} being presumably t^{w2}. This constitutes a sample of 19 alleles tested, none of them being t^{w1}. Added to the 13 alleles tested in 1955, this gives a total of 32 alleles tested in 1955 and 1959, none of which were t^{w1}. This gives an upper confidence limit on the proportion of the t^w alleles that were t^{w1} of 0.11 at the 97.5% level and 0.09 at the 95% level. Taking the frequency of t^w alleles in this population as approximately 0.37 (see below), this would give t^{w1} an upper frequency limit of about .04 or as many as 4 t^{w1} genes in 50 individuals. Nevertheless this is an

upper limit, and it is more likely that the t^{w1} allele is no longer in this population. We shall therefore treat the alleles found as t^{w2} and proceed to estimate their frequency in 1959 from table 1.

Before estimating the gene frequency, it is necessary to determine the transmission ratio, m, of t gametes from $+/t$ males. This can be done from the data in table 1 in two different ways. The most obvious way is to take the number of tailless offspring of these males, divided by the number of offspring (Brachy and tailless) carrying a *T*-gene from the mother. For 1959 this gives $m = 148/175 = 0.84571 \pm 0.027$, using only those males known to be heterozygous because they had at least one tailless offspring. This figure should be corrected for possible heterozygous males with no tailless offspring. Because of the high transmission ratio, there is a negligible chance of a male with no T/t offspring being heterozygous unless he has very few $T/-$ offspring. (It should be noted that in all studies of the transmission ratio and gene frequency of normal-tailed animals mated with $T/+$ animals, normal-tailed offspring, $+/t$ or $+/+$, give no information on the gene transmitted by the experimental animal, and only offspring that are $T/-$, Brachy or tailless, are informative.) In the present case, the corrected number of heterozygous males is found to be 23.003, due mainly to one male with two Brachy offspring, and the corrected total offspring is 175.005, giving a corrected $m = .084569$, a negligible change. For 1955 the figures are uncorrected $m = 94/112 = 0.8393 \pm 0.035$ and corrected $m = 94/112.206 = 0.8388$ with the same standard error. For both years combined, the corrected $m = 242/287.06 = 0.8430 \pm 0.0215$. The difference between 1955 and 1959 is 0.006 ± 0.044 and negligible.

This value differs significantly from the transmission ratio of 0.954 ± 0.011 derived from tests of male heterozygotes descended from the original samples of 1952 (Dunn and Suckling, 1956). The cause of the difference is unknown, but male transmission ratios are subject to variations causing heterogeneity in the ratios both between males and between litters from the same male (Dunn and Gluecksohn-Schoenheimer, 1939 and Dunn, unpublished), so that variances other than the binomial sampling variance are involved. For the present data heterogeneity between males giving at least one tailless offspring gives $\chi^2 = 33.4$ with 21 degrees of freedom, P just under 5% for 1955; $\chi^2 = 29.0$ with 22 degrees of freedom, $25\% > P > 10\%$ for 1959; and $\chi^2 = 62.7$ with 44 degrees of freedom, P = 3% for the combined data. Since there is then marginal evidence of heterogeneity, the transmission ratio was also calculated separately for each male and the mean and variance of these quantities calculated directly. The resulting values were $m = 0.881 \pm 0.048$ for 1955, $m = 0.860 \pm 0.032$ for 1959, and $m = 0.866 \pm 0.028$ for the combined data. For further computations in this paper, the round figure of .85 was chosen, which is intermediate between 0.842 for the total figures and 0.866 for the litter averages. The gene frequency calculations are relatively insensitive to small changes in this value. It is clear that this figure based on the *present data* and not the figure of .95 from the previous data must be used to analyze *these* data.

For the males, the gene frequency may be found to a close first approximation by counting. It is assumed that the population consists of three genotypes, t/t, $+/t$, and $+/+$. Since t/t males are sterile, we have:

u = the number of heterozygous $+/t$,

v = the number of homozygous $+/+$,

$r = v/(u+v)$ = frequency of $+/+$ ♂♂ in the fertile male part of the population,

q_{σ} = frequency of the *t*-allele in the entire male population.

Assuming the population to be in Hardy-Weinberg equilibrium (this assumption is dubious, see below), q_{σ} can be expressed in terms of proportions of the $+/t$ and $+/+$ genotypes in the population as

$$(1)\quad \frac{2q\,(1-q)}{2q\,(1-q)+2(1-q)^2}=\frac{q}{q+(1-q)}=q.$$

The corresponding expression in terms of the actual numbers would be

$$(2)\quad q_{\sigma}=\frac{u}{u+2v}\ .$$

For a first estimate u_1 is taken as the number of males having at least one tailless offspring and v_1 as the number of males having no tailless offspring. Then $r_1 = v_1/(u_1+v_1)$, and a first estimate of the frequency of t will be (with the values for 1955 from table 1) $q_{1_{\sigma}} = 23/(23+38) = 0.3770$. By the "delta method" or "law of propagation of errors" it can be shown that the approximate variance of the estimated q is

$$(3)\quad \sigma^2_{\sigma}=\frac{4r_1(1-r_1)}{(u+v)\ (1+r_1)^4}=.00563.$$

The first estimate, r_1, of the proportion of fertile males that are $+/+$ is too high, because in the single litter tested some $+/t$ males may not give any T/t offspring and will be counted as $+/+$. The probability that a fertile male is $+/+$ *and* has no T/t[w2] offspring is r. If the male has n offspring carrying the T-allele from the mother, the probability that the male is $+/t$ *and* has no T/t offspring is $(1-r)S^n$, where S is the probability of a $+$ gamete from a $+/t$ male. Here $S = 0.15$. The conditional probability that a male having n $T/+$ offspring and no T/t offspring is himself $+/t$ is then

$$(4)\quad \frac{(1-r)S^n}{r+(1-r)S^n}\ .$$

Unless n is 1 or 2 this probability is negligible, and accordingly any correction based on it will likewise be small. Taking this probability of misclassification into account and using the method of maximum likelihood, the corrected estimate is $\hat{q}_{\sigma} = 0.3777 \pm 0.075$ which differs only trivially from the uncorrected estimate of 0.3770. Because of the small difference, the previous standard error still applies.

TABLE 2. *Estimated gene frequencies assuming Hardy-Weinberg equilibrium (with H-W) and not assuming it (without H-W)*

		q	σ^2_q
1955♂	with H-W	.348 ± .069	.0047
" ♀	" "	.457 ± .050	.0025
1959♂	" "	.378 ± .075	.0056
" ♀	" "	.299 ± .049	.0024
1955 sexes comb.	" "	.419 ± .040	.00164
1959 " "	" "	.322 ± .041	.00165
all males	" "	.362 ± .051	.00256
all females	" "	.375 ± .035	.00122
all data	" "	.371 ± .029	.00082
1955♀	without H-W	.457 ± .051	.0025
1959♀	" "	.292 ± .049	.0024

Gene frequencies for females, $q_{♀}$, can be obtained similarly, except that since all three genotypes are fertile, the standard method of gene counting gives a first approximation directly. For 1955 this gives $q_{1_{♀}} = 0.535 \pm 0.047$. However for females the segregation ratio, S, above is no longer 0.15 but 0.5, and the correction for females that give only $+/+$ and $T/+$ or only $T/+$ and T/t offspring becomes more important. The method of maximum likelihood, assuming the Hardy-Weinberg equilibrium holds, here gives $\hat{q}_{♀} = 0.457 \pm 0.050$, which differs by more than one standard error from the uncorrected value.

Applying the same methods to both the 1955 and 1959 data, the values in table 2 are obtained. The gene frequency calculated from females in the 1955 sample seems out of line; the difference $q_{♀}$ (1955) − $q_{♀}$ (1959) is just significant at the 5 per cent level of significance ($t = 2.2$). However, none of the other possible differences is significant, and the four estimates of q give no significant heterogeneity, so it is legitimate to characterize this population by the weighted average frequency of the t-allele, 0.371 ± 0.029.

The estimates of q so far discussed depend on the assumption of a Hardy-Weinberg equilibrium in the population, and it will be shown below that agreement with these equilibrium frequencies is not to be expected. In the absence of the assumption,

TABLE 3. *Genotypic frequencies for females, observed, corrected for misclassification on the basis of gene frequencies estimated with and without the assumption of Hardy-Weinberg equilibrium, and theoretical frequencies for Hardy-Weinberg equilibrium for both sets of gene frequencies*

		q	t/t	$+/t$	$+/+$
1955 Observed			15	23	19
" Corrected	Est. with H-W	.457	11.9	28.0	17.1
" H-W Freqs.	" " "	"	11.8	28.3	17.0
" Corrected	Est. without H-W	.454	12.1	28.0	17.0
" H-W Freqs.	" " "	"	11.9	28.3	17.0
1959 Observed			6	17	23
" Corrected	Est. with H-W	.299	3.2	21.1	21.7
" H-W Freqs.	" " "	.299	4.1	19.3	22.6
" Corrected	Est. without H-W	.292	2.3	22.2	21.5
" H-W Freqs.	" " "	.292	3.9	19.0	23.1

the relative frequency, $1-r$, of $+/t$ among $+/t$ and $+/+$ males is estimated as before, but these two genotypes are no longer in the proportions of $2q(1-q):(1-q)^2$, and hence the gene frequency, q, in *all males* cannot be estimated. For females a modification of the maximum likelihood method, but with adjustments based on genotype frequencies, can be used.

Since the frequencies of the genotypes t/t, $t/+$, and $+/+$ add to one, they can be represented by two independent parameters. It is convenient to use q and a, giving genotype frequencies

$$(5)\quad qa + q^2(1-a),\ 2q(1-q)(1-a),\ (1-q)a + (1-q)^2(1-a)$$

respectively. Then q is the gene frequency of t, and a is a measure of departure from the Hardy-Weinberg equilibrium values. Any set of genotypic frequencies can be so represented: the largest possible a is $a = 1$, corresponding to no heterozygotes; $a = 0$ corresponds to Hardy-Weinberg equilibrium, and the algebraically smallest value of a is $-x/(1-x)$, where x is the smaller of q and $1-q$, corresponding to absence of the rarer homozygote. Formally a is the same as Wright's coefficient of inbreeding, but its interpretation is more general.

The estimates not assuming Hardy-Weinberg equilibrium (without H-W) are given in table 2, along with those which assume it (with H-W). For females of the 1959 sample, the estimates are $q = 0.299 \pm 0.051$, $a = -0.17 \pm 0.19$, and the correlation between the estimated q and a is -0.10. For females of the 1955 sample the corresponding values are $q = 0.457 \pm 0.050$, $a = +0.012 \pm 0.16$, correlation -0.06. For both years the estimates of q with and without H-W differ only negligibly compared with the standard errors. Furthermore, the measures of departure from Hardy-Weinberg equilibrium differ from zero by less than their standard errors, so there is no direct evidence of departure, *although the figures are compatible with considerable departures in either direction*. From the estimated q and a and the sample size and formula (5), a set of corrected genotype frequencies, taking account of heterozygotes that give only one kind of offspring, can be calculated. Table 3 gives the apparent genotype frequencies, the corrected frequencies, and the Hardy-Weinberg equilibrium frequencies for the q's estimated both assuming and not assuming Hardy-Weinberg equilibrium. Since this assumption has so little effect on the estimates of gene frequency in females for the present data, since the data are in such good agreement with the genotypic frequencies calculated on the assumption of the Hardy-Weinberg equilibrium, and since the gene frequency in males can be estimated only on the basis of some assumption about the Hardy-Weinberg equilibrium, the weighted average of the q values estimated with H-W for both sexes in both years, 0.371 ± 0.029, is taken as the best estimate of q.

(If we assume that $a = -0.166$ in 1959 males as it is in females, we can obtain an estimate of $q_{\sigma} = 0.32$, compared with 0.378 for $a = 0$. For 1955 we would assume a nearly zero and q_{σ} would be unchanged at 0.348. The weighted average of all four q values would be little affected.)

Theoretical Equilibrium Gene Frequency

Prout (1953) and Bruck (1957) have given expressions for the equilibrium gene frequency of t-alleles under various assumptions. None of their expressions covers the case of t^{w2}, for which the homozygote t^{w2}/t^{w2} is viable, but is sterile in males. We will now consider such a gene for which no other selective forces operate. Let the frequency of t be q_f in the eggs and q_m in the sperm actually contributing to the next generation. Note q_m is not the same as q_{σ}, the gene frequency in all adult males (both sterile and fertile). Then the zygotic frequencies in both sexes are $q_m q_f$ t/t, $q_m(1-q_f) + (1-q_m)q_f$ $+/t$, and $(1-q_m)(1-q_f)$ $+/+$. These values will not agree with the Hardy-Weinberg equilibrium if $q_m \neq q_f$. The gene frequency in the zygotes will be $q = (q_m + q_f)/2$, and in the absence of selection this will be q_f', the frequency of t in female gametes of the next generation. At equilibrium then, $q_f' = q_f$, $(q_m + q_f)/2 = q_f$, and $q_m = q_f$. Thus after equilibrium has been reached, and only then, Hardy-Weinberg frequencies will hold. To find the actual equilibrium value we now look at the frequencies in males. At equilibrium $q = \bar{q}$ and the genes are transmitted as follows. There are $\bar{q}^2$ t/t transmitting no genes, there are $2\bar{q}(1-\bar{q})$ $+/t$, each transmitting m t genes and $(1-m)$ $+$ genes, and there are $(1-q)^2$ $+/+$, each transmitting 1 $+$ gene. Thus there will be $2\bar{q}(1-\bar{q})m$ t genes and $2\bar{q}(1-\bar{q})(1-m) + (1-\bar{q})^2$ $+$ genes, giving

$$(6)\quad \bar{q} = \frac{2\bar{q}(1-\bar{q})m}{2\bar{q}(1-\bar{q}) + (1-\bar{q})^2}$$

$$= \frac{2\bar{q}m}{2\bar{q} + (1-\bar{q})} = \frac{2\bar{q}m}{1+\bar{q}} ,$$

and we have

$$(7)\quad \bar{q}(1+\bar{q}) = 2\bar{q}m ,$$

$$(8)\quad 1 + \bar{q} = 2m ,$$

and

$$(9)\quad \bar{q} = 2m - 1 ,$$

or

$$(10)\quad m = \frac{1+\bar{q}}{2} .$$

If $m \leq 1/2$, $\bar{q} = 0$ and the t-allele is lost. For a given $m > 1/2$, the equilibrium value of q is much larger than the corresponding value for lethal t-alleles,

$$(11)\quad \bar{q} = \frac{1}{2} - \frac{\sqrt{m(1-m)}}{2m} ,$$

given by Bruck (1957).

The difference in equilibrium frequency is due to selection against t/t homozygotes occurring in only one sex, and to including the sterile (but not lethal) t/t males in the calculation of gene frequency.

For $m = 0.85$ as in t^{w2}, the equilibrium value is $\bar{q} = 2(0.85) - 1 = 1.7 - 1 = 0.7$. This value is completely incompatible with the observed value in this study of $q = 0.37 \pm 0.03$. On the other hand if $\bar{q} = 0.37$, $m = 1.37/2 = 0.68$, which is far too low. We are thus led to the conclusion that either the population under study has not yet reached equilibrium, or there is some additional selection against the t-allele, probably in the form of reduced fertility or viability or both for one or more of the $+/t$ males or t/t or $+/t$ females. It is quite unlikely that after 15 years the population is still this far from equilibrium, and selection seems the more likely explanation. Another force that could also reduce the frequency of t is inbreeding; again this seems highly unlikely to be so strong in this particular population. A decision as to the mechanism acting would require additional data. In any event, the population will not be in Hardy-Weinberg

equilibrium unless the forces of selection, inbreeding, and different gene frequencies in eggs and sperm accidentally tend to nearly compensate one another, and it is for this reason that doubt was expressed in the previous section about the validity of the Hardy-Weinberg proportions.

The Effect of t^{w2}-type Alleles on Small Populations

Lewontin and Dunn (1960) considered the behavior of recessive lethal *t*-alleles in very small populations. They considered, for example, populations of 2 males and 6 females, and gave reasons for supposing they might be of biological importance. An electronic computer was used to generate such populations by random sampling and to record the results. With no selection except the lethality, no mutation, and various values of m, they found: (1) The *t*-allele must ultimately be lost from the population. (2) For $m = 0.95$, the half life of the *t*-allele in this size population was of the order of 50 to 100 generations. This half life was very sensitive to the value of m. (3) Unfixed populations most of the time had q between 1/4 and 1/2.

In generating these populations the size was kept constant by replacing any t/t zygotes (lethals) with viable zygotes. A natural population would do the same, since litters are large and frequent, lethals are resorbed in utero, and only a small proportion of offspring born reach maturity.

The situation for a viable *t*-allele with sterile male homozygotes is quite different. A sterile male zygote is not replaced, since in the absence of other selective forces it would compete on equal terms with its litter mates. In fact homozygous t^{w2} males seem quite normal in the laboratory, and are sexually active, producing normal copulation plugs. We can therefore imagine a t^{w2}/t^{w2} male as the "bull" of a "harem" of females, and driving off other males. However, such a family unit would have no offspring and would become extinct. Thus while the abnormal transmission ratio gives such a *t*-allele a strong advantage in intrapopulation selection, it has an equally strong disadvantage in interpopulation selection, at least in very small populations.

Thus a lethal *t*-allele can be lost from a small population by drift and the population will then remain with only the + allele until the *t*-allele is re-established by a migrant carrying a *t*-allele from a neighboring population. If such an immigrant is a male, Lewontin and Dunn have shown there is a considerable probability the *t*-allele will become temporarily established. A viable male sterile *t*-allele can also be lost from a population by drift, though this will happen less often; but in addition the allele may reach fixation in males, and exterminate the population. The resulting vacant niche will usually be quickly filled by mice from a neighbor population, quite likely carrying the *t*-allele. Other things being equal, such viable *t*-alleles might thus be expected to be more ubiquitous than the lethal alleles. On the other hand the wastage over large areas due to sterile males might make viable alleles so deleterious in interpopulation selection as to make these alleles less widespread than the lethals.

As an example of how such alleles behave, a table of random sampling numbers was used to generate a series of populations consisting of 2 males and 4 females. Each was started with 1 $+/t$ and 1 $+/+$ male, and with 2 $+/t$ and 2 $+/+$ females. With a transmission ratio $m = 0.85$, six populations reached the state of 2 t/t males (followed by extinction) in 4, 7, 7, 8, 9, and 14 generations. One population with $m = 2/3$ reached extinction in 12 generations. In none of the 7 "populations" did the +-allele reach fixation. While these few results show that there is a good chance of extermination in these small populations, many more samples would be needed to determine the relative chances of loss of the *t*-allele and of population extinction. However, on theoretical grounds it is clear that one of these two events must ultimately occur. If the number of males is fixed at 2 and the num-

ber of females is varied, the median time for extinction will be little affected, but the chance of elimination of the *t*-allele decreases rapidly as the number of females increases.

After reading this manuscript, Professor Lewontin modified his program for the 650 computer to take care of the present case. He has kindly given us permission to summarize his results as follows: Fifty-nine populations of 12 males and 12 females containing both *t* and + alleles rapidly reached a fluctuating equilibrium at *q* approximately 0.69, close to the theoretical 0.7. In no case was the + allele fixed before 100 generations, but in somewhat over half of the populations the *t*-allele was fixed in males before 100 generations, leading to extinction of the population, the estimated half life being 90 generations. Two hundred and twenty-one populations of 2 males and 4 females, initially consisting of 1 $t/+$ male, 1 $+/+$ male, 2 $t/+$ females, and 2 $+/+$ females were run to fixation. Fixation here was rapid, the half life being about 6 generations. About 30% of the population reached fixation with only $+/+$ individuals. Such populations would then be stable, with no *t*-alleles present unless reintroduced from outside. The remaining 70% reached fixation with only t/t males and would then become extinct. In this series, the mean gene frequency in unfixed populations (omitting the first six generations to eliminate the effect of the initial gene frequency) was about 0.5.

Summary

1. Two small samples in 1952 from a confined population of wild house mice showed the presence of two *t*-alleles, a recessive lethal, t^{w1}, and a viable with male sterility in homozygotes, t^{w2}.

2. Two larger samples were taken, one in 1955 including 43 fertile males and 57 fertile females, and one in 1959 including 42 fertile males and 88 fertile females. Analysis of 32 *t*-alleles recovered from these samples gave only t^{w2}, suggesting that t^{w1} is no longer present in this population.

3. While male $+/t^{w2}$ descendants of t^{w2} mice from the 1952 samples transmitted t^{w2} to about 95% of their offspring, $+/t$ males from the 1955 and 1959 samples had a transmission ratio of about 85%. This difference is statistically highly significant, but the reason for it is unknown.

4. The 1955 and 1959 samples gave an overall estimate of 0.37 for the gene frequency of the *t*-allele in adults, without significant heterogeneity.

5. The opposing forces of sterility of t/t males and a transmission ratio of 0.85 would lead to an equilibrium gene frequency of 0.70, suggesting the presence of other selective forces acting against *t* in the confined population.

6. Theoretical consideration of the behavior of genes like t^{w2} in a small population shows that there is a distinct possibility that at some generation all males will be t/t, and the population will then become extinct. Thus, other things being equal, such viable alleles are less disadvantageous than recessive lethal alleles in intrapopulation selection, but more disadvantageous in interpopulation selection.

Literature Cited

Bennett, D., L. C. Dunn, and S. Badenhausen. 1959. A second group of similar lethals in populations of wild house mice. Genetics, **44**: 795–802.

Dunn, L. C. 1939. The inheritance of tailessness (anury) in the house mouse. III. Tailessness in the balanced lethal line 19. Genetics, **24**: 728–731.

——. 1957a. Evidence of evolutionary forces leading to the spread of lethal genes in wild populations of house mice. Proc. Nat. Acad. Sci., **43**: 158–163.

——. 1957b. Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. Genetics, **42**: 299–311.

—— and S. Gluecksohn-Waelsch. 1953. Genetic analysis of seven newly discovered mutant alleles at locus *T* in the house mouse. Genetics, **38**: 261–271.

—— and W. C. Morgan, Jr. 1953. Alleles at a mutable locus found in populations of wild

house mice. Proc. Nat. Acad. Sci., **39**: 391–402.

—— AND JOAN SUCKLING. 1956. Studies of the genetic variability in wild populations of house mice. I. Analysis of seven alleles at locus *T*. Genetics, **41**: 344–352.

LEVINE, LOUIS, AND L. C. DUNN. 1956. Frequency of *t*-alleles in a confined population of wild house mice. Proc. Soc. Exp. Biol. Med., **92**: 308–310.

LEWONTIN, R. C., AND L. C. DUNN. 1960. The evolutionary dynamics of a polymorphism in the house mouse. Genetics, **45**: 705–722.

SCHNEIDER, H. A. 1946. On breeding "wild" house mice in the laboratory. Proc. Soc. Exp. Biol. Med., **63**: 161–165.

DIVERGENCE IN *TRIPSACUM* AND *ZEA* CHROMOSOMES[1]

MARJORIE P. MAGUIRE
Genetics Foundation, Department of Zoology, University of Texas

Received January 4, 1961

It is generally agreed that *Tripsacum* and *Zea* have probably evolved as divergent genera from an ancient common ancestor (Mangelsdorf and Reeves, 1939; Montgomery, 1906; Randolph, 1952; Weatherwax, 1918). The mode of origin of the striking differences between their chromosomes may be elucidated by studies of hybrid backcross progenies in which *Tripsacum* chromosomes or segments of them are included in a genetic background which is otherwise that of *Zea mays* L. (Mangelsdorf and Reeves, 1939; Maguire, 1957). The substitution of a large *Tripsacum* chromosome segment for a portion of the corn genome has made possible a direct comparison of the effects of corresponding corn and *Tripsacum* segments on the development and reproduction of the corn plant. The results of this study are reported here, with a consideration of the evolutionary processes which might have yielded the differences found between these two chromosome segments.

Divergent viewpoints on the relationship of *Tripsacum* and corn have been reviewed recently by Mangelsdorf and Reeves (1959) and Randolph (1959). The major differences between the two genera and their crossability are of importance to this study and will be outlined briefly below.

Diploid *Tripsacum* has 18 pairs of chromosomes with an average length much shorter than that of the 10 pairs of corn chromosomes, and the chromosomes of *Tripsacum* differ from those of corn in arm ratio and other features (Longley, 1941; Randolph, 1955). Corn and *Tripsacum* are phenotypically very different. *Tripsacum* has well-developed staminate and pistillate spikelets in all inflorescences, with the pistillate below on a distichous, disarticulating rachis. Corn normally has separate staminate and pistillate inflorescences with the staminate terminal, the pistillate lateral and enclosed by husks or modified leaves. The axis of the pistillate inflorescence of corn is polystichous and does not disarticulate. Growth habit, branching and leaf width are also very different in the two genera.

Artificial hybridization of corn and *Tripsacum* is difficult. When corn is used as the female parent, a few seeds (usually viable only under embryo culture) may be set following pollination with *Tripsacum* if the silks of corn have been shortened (Mangelsdorf and Reeves, 1939) or the pollen applied near the base of the corn silks (Randolph, 1955). Attempts to produce the reciprocal cross, using *Tripsacum* as the female parent, have failed except those of Farquharson (1957) who tested the crossability of *Tripsacum* with more primitive varieties of corn. She was able to obtain a hybrid plant which reached maturity from the pollination of *Tripsacum dactyloides* L. with a Peruvian stock of corn. Randolph (1952) found Mexican and Guatemalan *Tripsacum* and corn to be highly cross–incompatible. All the mature hybrids of corn and *Tripsacum* which have been produced have irregular meiosis, do not yield functional pollen, and the only functional eggs are those which contain the unreduced complement of 28 chromosomes. Maguire (1960) found genetic recombination to be extremely rare between corresponding corn and *Tripsacum* segments.

The genomic differences between the two genera and the difficulty of crossing them artificially support the view of Randolph (1955) and Weatherwax (1955) that recent natural exchange of chromosome material between corn and *Tripsacum* has probably happened rarely, if at all, with the possible

[1] This work was supported by a grant from the National Science Foundation (G-7068) and by a PHS research grant (RG-6492), from the National Institutes of Health, Public Health Service.

exception of South American forms (Farquharson, 1957).

Heterozygous and Homozygous Substitution Plants

Cytological Studies

The plants studied in this experiment contained a *Zea-Tripsacum* interchange chromosome in which the distal half of the short arm of corn chromosome 2 had been exchanged for a corresponding segment from a *Tripsacum* chromosome bearing a conspicuous terminal knob. Plants heterozygous for this chromosome (i.e., containing one interchange chromosome 2 and one normal chromosome 2) are referred to as "ZT" plants. Plants homozygous for this chromosome (two interchange chromosomes 2 present with complete exclusion of normal corn chromosome 2) are referred to as "TT" plants. Since the remainder of the complement of these plants comprises the rest of the corn chromosomes with no other apparent abnormalities or alterations, the letters "Z" and "T" in these designations refer only to the origin of the distal half of the short arm of chromosome 2. The T segment was contributed from the long arm of a chromosome derived from *Tripsacum* which was about half as long as corn chromosome 2 and had an arm ratio of about 3.3:1. The ZT plants were obtained from corn backcross progenies of *Zea mays* L.–*Tripsacum dactyloides* L. hybrids as described in earlier papers (Maguire, 1957, 1960); TT plants were segregants of intercrosses of ZT plants.

The T segment in ZT plants is thought to be intact as derived from *Tripsacum* (or at least not modified by crossing over), since these plants were the offspring of 21-chromosome plants containing both reciprocal interchange products and one normal corn chromosome 2. In such plants the two Z segments usually synapse, leaving the T segment univalent at pachytene. This failure of Z and T segments to synapse is considered evidence that the T segment was intact (or very nearly so) in these parent plants. The T segment is probably intact in TT plants, since recombination between Z and T segments in 20-chromosome plants was extremely rare in tests involving markers located relatively near opposite ends of the region in question (Maguire, 1960). No evidence has been seen in the thousands of clear microsporocytes studied (from plants of all the various constitutions) of other types of alteration of the Z or T segments such as inversion, translocation, deletion, or duplication. Minor aberrations which might not be detectable cytologically would not be expected to cause crossover inhibition of the extent found.

The T and Z segments bear a degree of mutual homology despite the failure of crossing over between them, as indicated by the following observations. In ZT plants the interchange and normal chromosomes 2 synapse completely and with no extension of one beyond the other in about 93% of microsporocytes (Maguire, 1960). In 21-chromosome plants containing two interchange chromosomes 2 (each with the T segment), and the reciprocal exchange chromosome (with the Z segment attached to the remainder of the original chromosome from *Tripsacum*), 10 bivalents and a univalent are found in about 95% of microsporocytes at pachytene. But in the rest of the cells a trivalent configuration is formed in which the Z segment of the extra chromosome synapses with one of the T segments to the exclusion of the other, which is assumed to match its partner perfectly or nearly so (fig. 1). Thus although there is preferential pairing of T with T, Z occasionally competes successfully in synapsis with T. Also, the T segment has been shown to carry normal dominant alleles for two recessives located in the distal half of the short arm of corn chromosome 2 (Maguire, 1960).

Phenotypic Studies

Plants of ZT constitution were obtained as described above, and these were compared with their ZZ sibs and with TT segregants. Plants were grown side by side from seeds planted simultaneously. In all cases the constitution of the plants was deter-

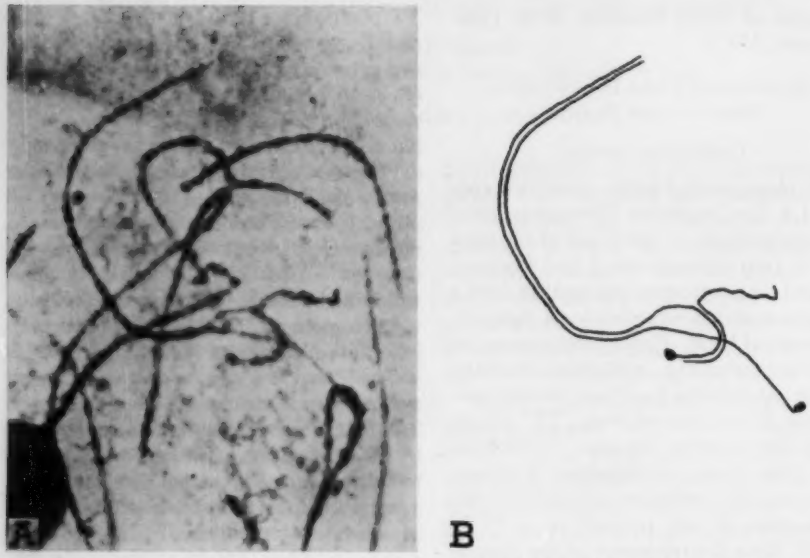


FIG. 1. A, Photomicrograph showing a trivalent configuration in a plant with two interchange chromosomes 2 (each with a T segment carrying a terminal knob) and an extra chromosome carrying a Z segment attached to *Tripsacum* chromosome material. This Z segment is synapsed with one T segment, while the other T segment is univalent. B, Semidiagrammatic representation of the trivalent configuration shown in A. Magnification about 1000 ×.

mined cytologically by means of presence or absence of the terminal knob of the T segment.

While ZT plants cannot be distinguished from ZZ plants on the basis of their gross appearance, TT plants have a characteristic conformation: They are short and stocky, with stiff leaves and very few tassel branches (fig. 2). Their silks are usually split (as illustrated in fig. 3) for an appreciable distance back from the tip, although this varies greatly from one plant to another (table 1). All available plants of ZT, ZZ, and TT constitution were measured and compared for a number of quantitative characteristics. The data are presented in table 2. Means and variances were calculated and *t* tests applied for an estimation of the statistical significance of differences of means. Because of the small numbers of plants involved in these tests and their probable genetic heterogeneity, the tests are insensitive to minor differences in effect of the Z and T segments, and are meaningful only where differences are striking.

The means of ZT and ZZ plants did not seem to differ from each other, but the means of both differed from the mean of TT plants in the following characteristics: TT plants were shorter, had fewer nodes, fewer tassel branches, a smaller number of rows of ovules, and a smaller number of ovules per row. Both ZT and TT plants showed a tendency to be proterogynous and to have narrow leaves. TT plants had more earshoots than ZZ plants, but ZT plants were intermediate in this respect, not differing from either ZZ or TT plants. ZT plants had longer leaves and more veins per centimeter of leaf width than either TT or ZZ plants. TT, ZZ, and ZT plants did not differ in number of tillers or internodal length.

It is difficult to evaluate the influence of substitution of the T segment on such characteristics as plant height (in this case

FIG. 2. Photograph of a typical TT plant.

FIG. 3. Photograph showing silks with split tips from an ear of a TT plant.

apparently a direct reflection of node number), number of rows of ovules, and number of ovules per row, since the differences correlated with substitution might result from a general reduction of vigor rather than direct effect of *Tripsacum* genes. However, the tendencies of TT and ZT plants to be more proterogynous and of TT plants to have more earshoots and split silks are probably most easily explained as tripsacoid influence. The drastic reduction in number of tassel branches in TT plants is probably also most easily explained in these terms.

Pollen fertility in ZT plants was normal, or very nearly so, with about 97% large, well-filled grains of uniform size. The proportion of pollen carrying the T segment which functioned in fertilization in direct competition with pollen carrying the Z segment from these plants was about 42%. TT plants showed a wide variation in proportion of shrivelled pollen. The proportion of normal-appearing pollen found in these plants ranged from 1% to 93%, with a mean of 73% and a variance of 37. This large variability of pollen in TT plants might result from a reduced tolerance to minor fluctuations from optimal conditions in plants of unbalanced genome, as well as to differences in the genotypes of the plants. Fabergé (1944) proposed such an explanation for variable fertility in his work with *Papaver* hybrids.

TABLE 1. *Lengths of split portions of silks in TT plants*

Number of silks measured	Mean length split (mm)	S^2
96	6.9	5.69
155	15.6	249.0
167	14.9	64.8
119	7.7	9.38
133	9.4	20.6

DISCUSSION

Perhaps the most surprising findings of these studies are that pollen with a T segment substituted for a Z segment is normal in appearance and function, and that TT plants are viable and fertile to a high degree. In other words it appears that a *Tripsacum* chromosome segment can be substituted for

TABLE 2. *Measurements of quantitative characteristics*

Characteristic	Chromosome constitution	Number of plants measured	$\bar{X}$	S^2
Proterogyny[1]	TT	23	+0.4	3.7
(dehiscence date	ZT	10	−0.3	0.9
minus silking date)	ZZ	12	−1.5	1.9
Plant height (m)[2]	TT	24	0.88[4]	0.027
	ZT	8	1.30	0.023
	ZZ	7	1.22	0.070
Number of nodes[2]	TT	24	6.7[4]	0.96
	ZT	8	9.0	0.86
	ZZ	7	9.7	0.83
Average	TT	24	13.3	8.3
internodal length	ZT	6	14.5	6.2
(cm)	ZZ	7	12.7	6.8
Number of tassel	TT	17	4.0[4]	2.0
branches[2]	ZT	9	12.9	2.37
	ZZ	7	12.1	5.50
Number of tillers	TT	25	0.44	0.34
	ZT	8	0.50	0.57
	ZZ	7	0.29	0.57
Number of	TT	25	2.2	0.67
earshoots	ZT	8	1.8	0.43
	ZZ	7	1.6	0.33
Number of rows	TT	20	9.4	2.58
of ovules[2]	ZT	7	10.8	5.67
	ZZ	6	11.6	2.20
Number of ovules	TT	20	22.8	22.1
per row[2]	ZT	7	30.7	13.5
	ZZ	6	27.8	31.0
Leaf length[3]	TT	23	58.9[4]	41.7
(cm)	ZT	8	85.9	75.4
	ZZ	7	76.8	55.2
Leaf width[1]	TT	22	6.8	0.43
(cm)	ZT	5	6.5	0.50
	ZZ	7	8.2	1.47
Vein number per	TT	22	1.7[4]	0.10
cm leaf width[3]	ZT	5	2.6	0.05
	ZZ	7	2.2	0.15

[1] Means of ZT and TT plants differ from mean of ZZ plants at 5 per cent level in *t* test.

[2] Means of ZT and ZZ plants differ from mean of TT plants at 5 per cent level in *t* test.

[3] Means of TT and ZZ plants differ from mean of ZT plants at 5 per cent level in *t* test.

[4] Mean differs from others at 1 per cent level in *t* test.

a corn chromosome segment equal to about 3% of the total length of the corn genome. Extensive deficiencies are rarely viable in corn gametophytes. To the writer's knowledge the most extensive haplo–viable deficiency was that reported by Stadler (1933) involving about ⅛ of the long arm of chromosome 10 (about 1% of the corn genome). While eggs carrying this deficiency were viable to some extent, pollen was not, and plants homozygous for it were never produced. It is probable that there are genes essential to normal development and reproduction of corn located in the distal half of the short arm of its chromosome 2, and that adequate substitutes for these exist in a *Tripsacum* chromosome region. Essential loci might be expected to be resistant to evolutionary change, but interchangeable arrays of such loci are not expected to exist in chromosome segments of equal length in genera whose genomes differ as widely as those of *Tripsacum* and corn. The question which naturally arises cannot be answered at present: Are the Z and T segments of this experiment unusual in their similarities, or are there widespread basic similarities between corn and *Tripsacum* chromosomes?

The exchange which produced the interchange chromosomes of this experiment was probably a crossover type of event, since it involved corresponding, partially homologous segments. It seems unlikely that it was terminal with respect to regions partially homologous, and unless the exchange was terminally located, at least an additional region of undetermined length must also correspond closely in the two genera. Unfortunately only the Z and T segments of this experiment have been tested so far for this kind of similarity, and further tests must await the development of strains with other interchange chromosomes. In view of the rarity of crossing over between these two regions other exchanges probably will not be readily obtained.

If strong similarities between corn and *Tripsacum* chromosomes are confined to a few well-delimited segments, the theory of Mangelsdorf and Reeves (1939) will be supported to the extent that recent exchange of chromosome material between corn and *Tripsacum* has been of importance, however rarely it may have occurred. But if simi-

larities of the sort demonstrated here are widespread, then chromosomes of *Tripsacum* must be basically more like those of corn than a comparison of their idiograms would suggest. There is evidence to support the view that 36 chromosome diploid *Tripsacum* actually arose as an allotetraploid (Randolph, 1955). In this case the basic genome of *Tripsacum* would be 9 chromosomes compared to the 10 of corn. The fact that corn chromosomes are much longer than those of *Tripsacum* at pachytene might be attributed in part at least to general differences in the development of the pachytene stage. Even different stocks of corn differ widely in the overall length of their chromosomes at pachytene, although the various members retain the same relative lengths with respect to each other. Is it conceivable that the differences between the basic corn and *Tripsacum* genomes may be traced to a few major and many minor chromosome aberrations? If so we must assume that the meiotic irregularities of the corn–*Tripsacum* hybrid are due to incompatibility factors, general unbalance, or possibly to synaptic or crossover barriers, rather than to wide differences in chromosome homology. Unfortunately, it has been impossible to date to prepare an analyzable pachytene slide from a corn–*Tripsacum* hybrid for direct study of synaptic relationships. Since crossover barriers exist between regions partially homologous, the failure to find bivalents at later meiotic prophase of the hybrid is inconclusive.

In any case chromosomal rearrangements must have been minor within the Z and T segments studied in this experiment. The differential changes responsible for their differing phenotypic effects, for the failure of crossing over between them and synaptic failure between them under some conditions must have been limited to change in non-essential loci. These non-essential loci available for differential change might have been already present in the common ancestral chromosome segment, or produced by duplication or rearrangement of essential loci in the course of divergence.

Summary

A segment from a *Tripsacum* chromosome has been substituted for the distal half of the short arm of corn chromosome 2 in plants whose chromosomes are otherwise apparently unaltered corn chromosomes. Plants both heterozygous and homozygous for this substitution have been obtained. Heterozygous plants are indistinguishable from normal corn in gross appearance, although they seem to differ somewhat in certain quantitative measurements. Plants homozygous for the substitution have a characteristic appearance, and differ more widely from normal corn in quantitative measurements. Both heterozygous and homozygous plants are vigorous and fertile. Since cytological and genetic evidence seem to support the view that the *Tripsacum* segment has remained intact, it is concluded that this segment probably contains all of the loci essential to the normal development and reproduction of the corn plant which are located in the distal half of the short arm of its chromosome 2. Processes are discussed which might have contributed to the similarities and differences of these corresponding segments in the two divergent genera.

Acknowledgment

The writer is grateful to Dr. L. F. Randolph for constructive criticism of the manuscript and helpful suggestions.

Literature Cited

Fabergé, A. C. 1944. Genetics of the Scapiflor section of *Papaver*. III. Interspecific hybrids and genetic homology. J. Genet., **46**: 125–149.

Farquharson, L. J. 1957. Hybridization in *Tripsacum* and *Zea*. J. Hered., **48**: 295–299.

Longley, A. D. 1941. Chromosome morphology in maize and its relatives. Bot. Rev., **7**: 263–289.

Maguire, M. P. 1957. Cytogenetic studies of *Zea* hyperploid for a chromosome derived from *Tripsacum*. Genetics, **42**: 473–486.

——. 1960. A study of homology between a terminal portion of *Zea* chromosome 2 and a segment derived from *Tripsacum*. Genetics, **45**: 195–209.

Mangelsdorf, P. C., and R. G. Reeves. 1939. The origin of Indian corn and its relatives. Tex. Agr. Exp. Sta. Bull., **574**: 1–315.

——. 1959. The origin of corn. II. Teosinte, a hybrid of corn and *Tripsacum*. Bot. Mus. Leafl. Harvard U., **18**: 357–387.

MONTGOMERY, E. G. 1906. What is an ear of corn? Pop. Sci. Monthly, **68**: 55–62.

RANDOLPH, L. F. 1952. New evidence on the origin of maize. Amer. Nat., **86**: 193–202.

——. 1955. History and origin of corn. II. Cytogenetic aspects of the origin and evolutionary history of corn. *In* Corn and Corn Improvement, ed. by G. F. Sprague: 16–61. Academic Press, New York.

——. 1959. The origin of maize. Ind. J. Gen. and Plant Breeding, **19**: 1–12.

STADLER, L. J. 1933. On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Missouri Agr. Exp. Sta. Res. Bull., **204**: 1–29.

WEATHERWAX, P. 1918. The evolution of maize. Bull. Torrey Bot. Club, **45**: 309–342.

——. 1955. History and origin of corn. I. Early history of corn and theories as to its origin. *In* Corn and Corn Improvement, ed. by G. F. Sprague: 1–16. Academic Press, New York.

COURTSHIP AND SEXUAL ISOLATION IN *DROSOPHILA PAVANI* BRNCIC AND *DROSOPHILA GAUCHA* JAEGER AND SALZANO[1]

SUSI KOREF-SANTIBAÑEZ AND EDUARDO DEL SOLAR O.[2]
University of Chile[3]

Received October 27, 1960

Within populations of a species, reproductive isolating mechanisms, including sexual isolation, may arise and be favored by natural selection if the populations have diverged sufficiently to make compromise genotypes unfavorable (Dobzhansky, 1944). Reproductive isolation, which may first be expressed at the sensorial level, may precede morphological differentiation (Spieth, 1952), and may manifest itself already at the racial or subspecific level. In an effort to determine the mechanisms of the establishment of reproductive isolation, several authors have studied incipient sexual isolation within species (Rendel, 1944; Dobzhansky, 1945; Patterson, Wharton and Stone, 1947; Smith, 1958; Koref-Santibañez and Waddington, 1958; Hoenigsberg and Koref-Santibañez, 1960, and others). In general, it has been shown that the rudiments of isolation within a species are genetically the same as those between species.

Courtship is the ritual which precedes mating, and the components of this ritual are so specific that they may be used as taxonomic characteristics (Spieth, 1952). Differences between groups which do not yet manifest themselves at the copulation level tend to appear as differential utilization of certain courtship elements, which may be explained by different stimuli thresholds on the part of either the male or the female (Hoenigsberg and Koref-Santibañez, 1959 a and b, 1960). Most of these studies were made on laboratory populations of cosmopolitan and domestic species such as *D. melanogaster*, or in wide-ranging wild species which form rather continuous populations such as *D. willistoni*, *D. pseudoobscura*, etc. How do these processes express themselves in more endemic wild species which live in restricted and isolated populations? In these latter it may be expected that the courtship behavior exhibited by each population will be more differentiated. For this reason, it was decided to study courtship and sexual isolation in members of the *mesophragmatica* group (Brncic and Koref-Santibañez, 1957). *Drosophila pavani* Brncic 1957 is a species belonging to this group, which lives exclusively in Chile and on the eastern slope of the Andes Mountains in Argentina. It forms a chain of rather small colonies which may be found from Copiapó (28° lat. S) to Valdivia (40° lat. S). These colonies are isolated from one another by mountain chains and semi-desert regions, which decrease or prevent interbreeding. *D. gaucha* Jaeger and Salzano 1953, another member of the same group, lives in southern Brazil and ranges up to western Argentina, in one region (San Luis) overlapping the distribution of *D. pavani*. These are sibling species which are difficult to distinguish morphologically, and although hybrids have never been found in nature they cross in the laboratory, giving sterile hybrids (Koref-Santibañez, Casanova and Brncic, 1958). Populations of *D. pavani* differ in the polygene contents of their chromosomes (Brncic, 1961). Therefore, these species are promising for the study of the appearance of behavioral differences which arise as by-products of the genetic divergence in different populations.

MATERIALS AND METHODS

The following stocks of flies were used in our study: five populations of *D. pavani*

[1] This work was done partly under Contract Number AT (30-1)-2465 from the U. S. Atomic Energy Commission.

[2] During the realization of this work E. del S. had a scholarship from "Comisión Inter-Facultades de Medicina."

[3] Instituto de Biología "Juan Noé," and Cátedra de Biología, Facultad de Filosofía y Educación. A. Zañartu 1042. Santiago. Chile.

TABLE 1. *Main courtship elements used by* Drosophila pavani *males from Chile with females of their own and of various other populations. The figures indicate numbers of times males used each element toward females of their choice.*

♂ *pavani* Chile	a ♀ *pavani* Chile + ♀ *pavani* Chile n = 430			b ♀ *pavani* Chile + ♀ *pavani* Mendoza n = 109			c ♀ *pavani* Chile + ♀ *gaucha* n = 228		
	Homogamic[1]	Heterogamic[2]	Chi-square	Homogamic[1]	Heterogamic[2]	Chi-square	Homogamic[1]	Heterogamic[2]	Chi-square
Orientation	696	536	20.76[3]	173	146	2.28	339	270	7.82[3]
Vibration	2,001	1,090	268.48[3]	416	352	5.34	767	670	97.00[3]
Circle	812	408	133.78[3]	161	133	2.66	250	242	0.12
Tap	1,375	694	125.44[3]	290	230	6.92[3]	527	416	12.06[3]
Lick	1,185	511	266.84[3]	232	194	3.38	400	430	1.08
Antennae	218	91	52.20[3]	36	29	0.76	76	55	3.36
Copulation	78	56	3.62	24	29	0.48	34	63	8.66[3]
Courtship time (minutes)	25,222	14,035	111.87[3]	5,546	5,417	1.28[3]	11,453	8,778	353.70[3]

n = number of males.
[1] = females of same population as males.
[2] = females of different population than males.
[3] = $p < 0.01$ with 1 degree of freedom.

from Chile: Copiapó (in the northern part of the country), Arrayań and Viña (in the central part), Los Queñes and Chillán (in the southern part of the country); one population of *D. pavani* from Mendoza, Argentina; and two populations of *D. gaucha*, one from Taimbas, Brazil, and the other from Córdoba, Argentina. All these populations had been maintained in the laboratory for from two to four years.

Flies were taken within a few hours of hatching, so males and females had not mated. They were kept separately at a constant temperature of 25° C for about 10 days, a period after which the sexual activity is at its maximum.

To detect courtship discrimination between populations, the male choice method (Dobzhansky and Mayr, 1944) was used. One male from each population was placed in an empty vial together with two females, one from the same strain as the male and the other from a different strain. Twenty-four hours before the observation one of the females was marked with a spot of silver paint on her thorax, to distinguish her from the other female in the same vial. Direct observations were made of courtship behavior in empty 1 × 3 cm vials, over a period of 30 minutes. The experiments were carried out during the morning hours, at room temperatures of about 22°–23° C. Courtship behavior was analyzed and recorded according to the nomenclature and methods described by Spieth (1952) and Hoenigsberg and Koref-Santibañez (1960).

RESULTS

Courtship rituals in *D. pavani* and *D. gaucha* are in general very similar to those in other species of the subgenus *Drosophila* (Spieth, 1952), with perhaps a greater utilization of contact stimuli such as tapping and licking, although vibration is also used quite profusely. A more detailed analysis of the courtship behavior will be presented elsewhere. In about 40% of the observations no courtship activity was seen during the 30-minute observations. These were discarded, and the data involving a total of 1,240 males were analyzed.

Although there are individual variations in the activity of each population, the overall tendency may be summarized in the results shown in tables 1, 2, and 3.

The behavior of *D. pavani* males from Chile is recorded in table 1. Tapping, vibration, and licking were most frequent; these males are also the most discriminating, more frequently courting females of their

TABLE 2. *Main courtship elements used by* Drosophila pavani *males from Mendoza (Argentina) with females of their own and of various other populations. The figures indicate numbers of times males used each element toward female of their choice.*

♂ *pavani* Mendoza	a ♀ *pavani* Mendoza + ♀ *pavani* Chile n = 110			b ♀ *pavani* Mendoza + ♀ *gaucha* Cordoba and Taimbas n = 51		
	Homogamic	Heterogamic	Chi-square	Homogamic	Heterogamic	Chi-square
Orientation	152	91	15.32[1]	74	52	3.84
Vibration	358	182	57.38[1]	124	86	6.88[1]
Circle	157	40	69.48[1]	42	35	0.64
Tap	256	90	79.64[1]	102	41	26.02[1]
Lick	228	91	58.84[1]	64	38	6.62[1]
Antennae	48	8	14.29[1]	13	4	5.76
Copulation	35	12	11.26	16	9	1.96
Courtship time (minutes)	3,585	1,368	2,758.3[1]	1,415	711	233.12[1]

n = number of males.
[1] = $P < 0.01$ with 1 degree of freedom.

own population (homogamic column) than foreign females (heterogamic column). Although *D. pavani* males spent significantly more time courting females from their own group, they did not mate with them more frequently (section a, table 1). *D. pavani* males from Chile, when confronted with females from Chile and Mendoza (section b, table 1), showed practically no discrimination. Only tapping was used slightly more towards their own females. *D. pavani* males (section c), although courting preferentially the homogamic females, inseminated more *D. gaucha* females than *D. pavani* females.

Table 2 shows that *D. pavani* males from Mendoza discriminate quite intensely against *D. pavani* females from Chile (section a). These males use all the elements of courtship significantly more toward their own females. With *D. gaucha* females, on the other hand, the preferential tendency is less marked, although present. In copulation, in contrast to what is observed with Chilean males, there is only a slight tendency for heterogamic mating.

Table 3 summarizes the observations on the activity of *D. gaucha* males. These exhibit practically no interpopulational preferences (sec. a), nor do they show any preferences between females of their own population and *pavani* females from Mendoza (sec. c). On the other hand, when confronted with *D. pavani* females from Chile these males direct the greater part of their activity toward their own females, also mating with them in a significantly higher proportion.

Although *D. gaucha* males use most of the elements of courtship in a fashion similar to *D. pavani* males, they make less use of the act of antennae touching.

In both species, the copulatory periods last about 50–60 minutes, and although, in general, this process is shorter in heterogamic copulations, their difference is not significant.

DISCUSSION

The results briefly analyzed above indicate that in *D. pavani* certain variations have arisen which express themselves as courtship preference of males toward females of their own population, although this preference does not reach the mating level. This is especially clear in the behavior of individual populations: the more distant ones, as for instance Copiapó and Los Queñes or Chillán, separated by strong mountain barriers, show the highest discrimination. Therefore, these populations, geographically isolated, with different ge-

TABLE 3. *Main courtship elements used by* Drosophila gaucha *males with females of their own and of various other populations. The figures indicate numbers of times males used each element toward females of their choice.*

♂ *gaucha*	a ♀ *gaucha* + ♀ *gaucha* n = 42			b ♀ *gaucha* + ♀ *pavani* Chile n = 230			c ♀ *gaucha* + ♀ *pavani* Mendoza n = 228		
	Homogamic[1]	Heterogamic[2]	Chi-square	Homogamic[1]	Heterogamic[2]	Chi-square	Homogamic[1]	Heterogamic[2]	Chi-square
Orientation	47	46	0.010	374	219	40.52[3]	55	60	0.22
Vibration	110	86	2.94	644	343	91.80[3]	84	106	2.54
Circle	18	29	2.58	204	138	12.74[3]	29	38	1.20
Tap	72	46	5.72	426	239	52.58[3]	58	43	2.22
Lick	62	40	4.74	417	163	111.24[3]	47	55	0.58
Antennae	4	4	—	30	22	1.22	5	3	0.50
Copulation	9	15	1.5	94	18	51.56[3]	7	10	0.52
Courtship time (minutes)	904	702	29.92[3]	6,324	3,403	877.16[3]	951	853	5.32

n = number of males.
[1] = females of same population as males.
[2] = females of different populations than males.
[3] = $P < 0.01$ with 1 degree of freedom.

netic constitutions (Brncic, 1961), have also evolved, together with the processes of divergence of their genetic systems (Muller, 1949), incipient barriers to genetic exchange. Many of these preferences are of the "one-sided" type (Dobzhansky, 1944), in which females of a population A discriminate against males of population B, but females of population B do not discriminate against males of population A (*D. pavani* from Chile, and *D. gaucha* females, respecting *D. gaucha* males). As stated by Dobzhansky (1944) and Merrel (1949), among others, this type of preferential mating may initiate a trend towards a greater divergence.

Why is no sexual isolation observable between such "good" species as *D. gaucha* and *D. pavani*? Dobzhansky and Koller (1938) and Mayr (1946) have described the existence of less marked isolation between certain species of the *obscura* group, with increasing distance between the populations considered. Sympatric populations, on the other hand, exhibit the greatest discrimination. A similar situation seems to occur in our species, which are sympatric only in a restricted region in Argentina. Therefore, natural selection has not had an opportunity to intensify the barriers to cross-fertilization. It has only acted to lower the stimulus-response threshold of *D. gaucha* females.

What is the situation in the populations of *D. pavani* from Argentina, relatively near the region where both sibling species overlap? Toward the Chilean populations, both males and females of Mendoza origin act in a way similar to males and females from the populations of Chile. Nevertheless, Chilean males exhibit less activity and discrimination than do males from Mendoza. With *D. gaucha*, on the other hand, the barrier is more marked, Mendoza males showing both courtship and mating preferences.

In general, there is a kind of gradient of sensory response in these populations and species, which may reflect their phylogenetic relationships. This gradient is revealed especially in the receptivity of the female, which in polygamous forms such as *Drosophila* is the real discriminative partner which ultimately decides whether mating will occur or not (Smith, 1958). *D. gaucha* females are the most receptive, with the lowest stimulus-response threshold; there follow *D. pavani* from Mendoza, and finally *D. pavani* from Chile, with a very high threshold of response. This observation confirms a previous study of Brncic (1958), which showed that *D. pavani* fe-

males are inseminated at a lower rate than *D. gaucha* females in heterogamic crosses.

Within the species studied there are elements which may lead to a reduction of the gene exchange between different geographically isolated populations. If these populations were to meet, and the hybrids were at a disadvantage, these differences could become more intense (Dobzhansky, 1941; Koopman, 1950; Knight, Robertson, and Waddington, 1956). If, on the other hand, the hybrids were to show a high fitness, the differences will eventually be swamped out. The one-sided mating preferences between *D. gaucha* and *D. pavani* from Chile are probably due to their allopatric condition, but still suggest the existence of sensory preferences (Hoenigsberg and Koref-Santibañez, 1960).

Summary

An analysis by means of the male choice method was made of the courtship behavior of five populations of *D. pavani* from Chile, one population of *D. pavani* from Mendoza, Argentina, and two populations of *D. gaucha*, one from Brazil and another from Argentina.

Within the Chilean populations of *D. pavani* there are homogamic courtship preferences—greater frequencies of certain elements of courtship and longer courtship time with females of the same population as males. These homogamic courtship preferences do not result in greater frequencies of homogamic matings.

D. gaucha males exhibit one-sided mating preferences when confronted with *D. gaucha* females and *D. pavani* females from Chile.

D. pavani males from Mendoza (Argentina) discriminate more against *D. gaucha* females than do *D. pavani* males from Chile. This may be a consequence of the sympatric condition of these species in Argentina.

Acknowledgments

The authors wish to thank Dr. D. Brncic for his help and encouragement during the realization of this work, and for his valuable suggestions in the preparation of the manuscript. They wish to express their appreciation to Prof. T. Dobzhansky for his kind help in the revision of the manuscript, and to acknowledge the technical aid of Miss M. D. Pellicer.

Literature Cited

Brncic, D. 1958. The *mesophragmatica* group as an example of the speciation phenomena in *Drosophila*. Proc. X. Int. Cong. Gen., **1**: 420–433.

——. 1961. Integration of the genotype in geographic populations of *Drosophila pavani*. Evolution, **15**:92–97.

—— and S. Koref-Santibañez. 1957. The *mesophragmatica* group of species of *Drosophila*. Evolution, **11**: 300–310.

Dobzhansky, T. 1941. Genetics and the origin of species. Columbia Univ. Press, New York.

——. 1944. Experiments on sexual isolation in *Drosophila*. III. Geographic strains of *D. sturtevanti*. Proc. Nat. Acad. Sci., **30**: 335–339.

—— and P. C. Koller. 1938. An experimental study of sexual isolation in *Drosophila*. Biol. Zentr., 589–607.

—— and E. Mayr. 1944. Experiments on sexual isolation in *Drosophila*. I. Geographic strains of *D. willistoni*. Proc. Nat. Acad. Sci., **30**: 238–244.

Hoenigsberg, H. F., and S. Koref-Santibañez. 1959. Courtship behavior in inbred and outbred lines of *Drosophila melanogaster*. Ist. Lomb. Sc. Lett. (Cl.Sc), **93**: 3–6.

——, S. Koref-Santibañez, and G. P. Sironi. 1959. Incipient intraspecific sexual isolation in *Drosophila prosaltans* Duda and in *Drosophila equinoxialis* Dobzhansky. Experientia, **15**: 223.

—— and S. Koref-Santibañez. 1960. Courtship and sensory preferences in inbred lines of *Drosophila melanogaster*. Evolution, **14**: 1–7.

Knight, G. R., A. Robertson, and C. H. Waddington. 1956. Selection for sexual isolation within a species. Evolution, **10**: 14–22.

Koopman, K. F. 1950. Natural selection for reproductive isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Evolution, **4**: 135–148.

Koref-Santibañez, S., and C. H. Waddington. 1958. The origin of sexual isolation between different lines within a species. Evolution, **12**: 485–493.

——, A. Casanova, and D. Brncic. 1958. Experiments on sexual isolation in species of the *mesophragmatica* group of *Drosophila* (Diptera). Proc. R. Ent. Soc. Lond., (A) **33**: 179–185.

Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. III. The nature of the isolating mechanisms between *D. pseudoobscura* and *D. persimilis*. Proc. Nat. Acad. Sci., **32**: 128–137.

—— AND T. DOBZHANSKY. 1945. Experiments on sexual isolation in *Drosophila*. IV. Modification of the degree of isolation between *Drosophila pseudoobscura* and *Drosophila persimilis* and of sexual preferences in *Drosophila prosaltans*. Proc. Nat. Acad. Sci., **31**: 75–82.

MERREL, D. J. 1949. Selective mating in *Drosophila melanogaster*. Genetics, **34**: 370–389.

MULLER, H. J. 1949. The Darwinian and modern concept of natural selection. Proc. Amer. Phil. Soc., **93**: 459–470.

PATTERSON, J. T., L. WHARTON MCDONALD, AND W. S. STONE. 1947. Sexual isolation between members of the *virilis* group of species. Univ. Tex. Publ., **4720**: 7–31.

RENDEL, J. M. 1944. Genetics and cytology of *Drosophila subobscura*. II. Normal and selective mating in *D. subobscura*. J. Genetics, **46**: 287–302.

SMITH, J. M. 1958. Fertility, mating behaviour and sexual selection in *D. subobscura*. J. Genetics, **54**: 261–279.

SPIETH, H. T. 1952. Mating behavior within the genus *Drosophila*. (Diptera). Bull. Am. Mus. Nat. Hist., **99**: 401–474.

GALÁPAGOS TOMATOES AND TORTOISES

CHARLES M. RICK AND ROBERT I. BOWMAN[1]
University of California, Davis, and San Francisco State College

Received November 22, 1960

One of the several unique features of tomatoes native to the Galápagos Islands—*Lycopersicon esculentum* var. *minor* (*L. cheesmanii* var. *minor*) and related forms—is seed dormancy. Less than one per cent of their seeds, which are notably smaller than those of other known tomatoes, will germinate without treatment. Germination of up to ten per cent can be obtained with var. *minor* if part of the seed coat is carefully excised (Rick, 1956). The present report deals with attempts to break the seed dormancy and, particularly, to find mechanisms in the native environment that might account for germination and establishment there of the Galápagos tomatoes.

Recently, improved germination of these forms was sought by trying methods that are familiar to horticulturists for their effectiveness in breaking seed dormancies of various plant species. We found that soaking seeds for various periods in stagnant or well-aerated water, subjection of dry or wet seeds to various temperatures or combinations of temperatures, and acid treatments were ineffective in raising emergence percentages. Following the discovery by Benedict and Robinson (1946) of the improved germination of guayule seeds effected by sodium hypochlorite treatments and successful applications of this method by Taylor (1949) to celery and by Laude (1951) to smilo grass, we tested its effectiveness on seeds of LA166, one of the most refractory of our Galápagos collections. Four lots were started: (1) control; (2) seed coats partly excised; (3) one-hour soaking in 2.6% sodium hypochlorite solution(half-strength household bleach); (4) soaking for two hours in the same strength solution. Seeds of treatments 3 and 4 were thoroughly rinsed after exposure to hypochlorite. Germination was tested on moist blotting paper kept in plastic sandwich boxes. Treatment 3 removed most of the seed coat, partly exposing endosperm and embryo, while treatment 4 removed even more of the tissues. Most of the emergence occurred in the first eight days after sowing. After 15 days, the per cent germination in each lot was as follows: (1)—0, (2)—24, (3)—71, (4)—8. It is evident from these results and from the appearance of treated seeds that damage had resulted from the two-hour treatment (4). It has been our recent experience with certain other Galápagos accessions that seeds which fail to respond to a one-hour treatment may be induced to germinate by repeated treatments of half-hour and one-hour duration. Seedlings have been observed literally to pop out of the seed coats within minutes after these repeated treatments as if they had been simply unable to break through the seed coats. The one-hour treatment has become a standard procedure in our work and has continued to effect marked improvement in germination of the Galápagos accessions and also collections of the tomato species *L. peruvianum* and *L. chilense* as well as of the nightshades, *Solanum pennellii* and *S. lycopersicoides*.

Although the hypochlorite treatment provides a neat solution to the problem of germinating Galápagos tomato seeds, it does not solve the problem of establishment in nature. Various forms of these tomatoes exist in a wide distribution over the archipelago. Though not very abundant in most populations, their distribution extends to all of the larger islands and to several localities on each as verified by our observations and by earlier collections documented in herbarium specimens. We were not able to find them in certain areas (for example,

[1]This work was supported in part by grant GA-AGR-5547 of the Rockefeller Foundation. We are also grateful for the able assistance of Dora G. Hunt in the germination tests.

James and Hood Islands) from which they had previously been taken, but their absence can readily be explained by the plague of feral goats that infest these islands.

In the course of these investigations it has been possible to test a number of mechanisms that might account for germination of the dormant seeds. Visits to the islands in 1952–53 and 1957 by RIB and in 1956 by CMR provided opportunity for making the following field observations. The great majority of the living plants are large, and it is evident from the extent of their growth that they are fairly long-lived perennials. Occasional small plants could be found in the crevices between the ubiquitous lava rocks, but their presence gave no clues as to the mode of their establishment.

The following two tomato accessions were selected for germination tests: LA166, *L. pimpinellifolium*, collected on Indefatigable Island ½ km from Academy Bay along the trail that leads from the coast to the farms in the highlands; LA317, *L. esculentum* var. *minor*, collected on Bartholomew Island about 200 m from the anchorage on the north shore. These collections, representing the two dominating biotypes in the archipelago, are described in detail by Rick (1956).

To test the possibility that some substance or microorganism in the native soil might facilitate germination, the following test was made in 1956. In a single container of soil collected under plants at the site of the LA166 collection were planted 100 seeds each of (1) freshly extracted seeds of a large horticultural tomato (*L. esculentum*) grown in a nearby garden, (2) seeds immediately extracted from a plant of LA166, (3) seeds similarly obtained from another plant of LA166, and (4) seeds from the plant sampled in lot 2 that had been previously extracted and dried on paper. The soil was moistened by rain water previously collected in a cistern. For the 15 days of the test, the soil was maintained at various moisture levels. The only lot to germinate was No. 1, of which 38 seedlings had emerged by the sixth day. Mr. Alf Kastdalen, a farmer-naturalist on Indefatigable Island, later made repeated unsuccessful attempts, some continuing over much longer periods, to start seeds from the same population. In view of these results it appears highly unlikely that any agent in the Galápagos soils stimulates germination.

Rumination Tests

Attention was paid to any possible relationships between the Galápagos tomatoes and native animals in all the observed populations. In most of the colonies no evidence could be found of animals feeding on the fruits. Ripe fruits were retained on the plants indefinitely, eventually drying to the consistency of raisins. Various species of Darwin's finches (*Geospiza*), and particularly the Galápagos Mockingbird, *Nesomimus parvulus parvulus*, were seen frequently in the vicinity of the tomato populations. They were observed to visit the tomato plants, but in no instance did they display interest in the ripe fruits, which were always abundant. Seeds positively identified as those of Galápagos tomatoes were nevertheless found in crops and gizzards of *Geospiza fortis*, *G. fuliginosa*, *Camarhynchus psittacula*, *C. parvulus*, and *Platyspiza crassirostris* collected on Indefatigable Island.

Evidence of animal feedings on native tomatoes was encountered in two colonies—one on Chatham Island near Wreck Bay, the other on Bartholomew Island, the site of LA317. In both populations fragments of fruits and droppings containing seeds and pieces of fruit epidermis revealed that the tomatoes were being eaten by some species of rodent. Dr. Seth B. Benson of the Museum of Vertebrate Zoology, University of California, Berkeley, kindly examined the pellets and identified them as the excrement of a rat species, probably introduced *Rattus* and not likely the native rice rat, *Oryzomys*. Many of the seeds in the pellets were damaged, most likely as a result of mastication. Numerous intact seeds were extracted from the pellets, washed, and planted on blotting paper, but none germinated.

An exploratory test was conducted to

ascertain what effects bird digestion might have on viability of these seeds. Week-old chicks of the domestic fowl were fed counted lots of LA166 seeds, all droppings were subsequently collected, and seeds were extracted from the droppings. We are indebted to Dr. F. H. Kratzer of the Department of Poultry Husbandry, University of California, Davis, for handling the feeding and collection phases of this test. Somewhat less than five per cent of the seeds were recovered in an intact condition, the remaining being detected as empty seed coats, broken portions of endosperm and embryo, or fragments so completely demolished that they could not be identified. None of the intact seeds germinated. In view of these results and those of Roessler (1936; see discussion) with the California linnet, it is unlikely that species of *Geospiza* or any other bird with a gizzard plays a role in the inducement of germination or dispersal of Galápagos tomatoes.

Reptiles constitute a peculiarly large proportion of the Galápagos fauna. Of these the giant tortoises have been a dominant element in actuality as well as in reputation of the archipelago. Since tortoises are notoriously fond of fresh fruit and vegetables, it might logically be suspected that they play a role in the germination and dispersal of tomato seeds.

An opportunity for testing the effects of tortoise digestion arose when RIB returned from the islands in 1957 with two young specimens of *Testudo elephantopus porteri* from Indefatigable Island. In the subsequent two years they have grown rapidly in small compounds of lawn in Berkeley, California. Temperatures of this environment are generally lower than those of the native habitat. The specimens tend to remain under cover during cool periods and to emerge and become more active only in warmer weather. At the time of the following tests they weighed 11½ and 26 pounds apiece.

Ingestion of the seeds presented no difficulties since the tortoises proved to be veritably indiscriminate eaters. This objective was attained simply by sprinkling the tomato seeds in the fashion of a condiment on lettuce and other items of their diet. In three of the feeding tests a liberal amount of carmine dye was fed simultaneously as a marker for identifying the feces that might contain seeds. The tortoises were maintained on a varied diet, the only items avoided being those that might be confused with the ingested tomato seeds. They were permitted to roam within their compound, where they could also feed on grass and soil materials. After the feedings the feces were collected at daily intervals, dried in the sun, and wrapped for temporary storage. Seed extractions were attempted after all feces of a single test had been collected. By a combination of washing, sedimentation, screening and flotation, the tomato seeds could be readily separated from other components. The effectiveness of this method is revealed by the rate of recovery of the seeds. The seeds were then counted, dried, and stored in packets for subsequent germination tests. For the emergence tests reported here the period of storage never exceeded one day.

Germination of the seeds was tested on blotting paper kept in plastic sandwich boxes. The paper was moistened with tap water and the boxes were placed in a well-lighted situation at room temperature, which varied from 25° to 30° C. The seeds were counted and removed as they sprouted. Numerous seedlings were later transferred to soil in greenhouse cultures, where nearly 100% survived and grew with normal vigor. In view of such successful establishment, the observed sprouting can be justifiably equated with germination.

For the first test a large lot of several thousand seeds of LA166 was fed to the tortoises in early April, 1960. No seeds were recovered from the feces, which had been collected for a two-week period following feeding. It was apparent from the remarkably intact condition of various ingested plant parts, including seeds of 12 different species, that such tortoise digestion would not likely damage the tomato seeds. Although two weeks is a remarkably long

TABLE 1. *Seed recovery data for second tortoise feeding test*

Days after feeding	Number of feces	Carmine in feces	Number recovered seeds		
			Normal	Damaged	Total
9	1	None detected			
11	4	Present	9		9
12	2	Present	2		2
13	3	Present	124		124
14	5	Present	714	7	721
15	1	Present			
16					
17	8	Present in few	76	2	78
18	7	Present in 2	86		86
20	4	None detected	13		13
		Total			1,133

Approximately 4,620 seeds of LA317 fed to tortoises June 18, 1960.

period for complete passage of a digestive tract, the only reasonable explanation for the failure of recovery was that a longer period was required and that feces containing the fed seeds would have been evacuated subsequent to the two-week period.

For the second test, seeds of LA317 were fed to the tortoises in late June, 1960. The seed number for this feeding was estimated by weight to be 4,620. Carmine was fed simultaneously as a means of marking the feces most likely to contain seeds. The dye did not appear in the feces until the 11th day after feeding, on which date tomato seeds also first appeared. As revealed in table 1, feces continued to be tinged with the dye until the fifteenth day; thereafter carmine was not in evidence in the feces or in the water in which they were soaked for seed extractions, but coats of some of the seeds appearing at later dates were strongly pigmented with carmine. Seeds continued to appear in the droppings until the 21st day —the final day of the collections. Judging from the rate of seed recoveries, a few more would probably have been obtained had collections been continued beyond that date. Less than one per cent of the recovered seeds showed any sign of damage detectable by use of a low-power dissecting microscope. A total of 1,133 seeds, or 24.5% of the number fed, was recovered.

Other contents of the tortoise excrement should be mentioned here as they might be of general interest. In all the tests, a weird assortment of items was recovered. The dominant element was grass leaves, which, despite digestion, could readily be recognized. Pine needles were usually present, sometimes comprising most of the content of the droppings, and compressed into tight, fibrous pellets. A small proportion of sand and gravel with particles up to one centimeter in diameter was always present. A predilection for large, sharp, coarse objects was evident in the recovery of walnut shell fragments, acorns, and small pieces of wood. Rubber bands and safety pins were also ingested. The ability of the Galápagos tortoises to pass such items is not surprising in view of their diet of *Opuntia* pads, spines included, during the dry season in their native habitat.

Seeds extracted from excrement collected 14, 17, and 19 days after feeding were subjected to germination tests. Untreated seeds, from the same source as those which were fed to the tortoises, served as controls. Also included were lots that had been soaked anaerobically in water for seven weeks and one that was treated for one hour in the dormancy-breaking hypochlorite solution. Each lot, except for the last mentioned, was subdivided into two equal groups, one being planted directly, the other being subjected to the hypochlorite solution for five minutes before planting. The latter treatment was applied as a sterilization precaution, which later tests revealed to be unnecessary.

Results of this germination test are presented in table 2. The point of primary interest is the very great improvement in germination wrought by tortoise digestion. Whereas one per cent germination was observed in the untreated control, comparable lots of recovered seeds germinated at the rates of 81, 80, and 60 per cent. The sterilization treatment generally increased the percentage emergence, but the differ-

TABLE 2. *Germination of seeds recovered in second tortoise feeding test and those of other treatments and control*

Treatment	Steril-ization[1]	Total planted	Number of seeds: Days after planting 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Germinated: Number	%
Control		100				1												1	1
	treated	100		1		1		1		2	2	1		2				10	10
42 days in water		180										1						1	0.5
Hypochlorite[2]		142		6	22	8		24	28	5	5	3						101	81
14 days in tortoise		100		58	14	7	1				1							81	81
Same	treated	100	4	75	13			1		1			1					95	95
17 days in tortoise		39		10	15	3		1		1	1							31	80
Same	treated	38		14	6	1	1			1	1							24	63
18 days in tortoise		45		18	2			1		3	3							27	60
Same	treated	40	2	24	3	1	1											31	77

[1] 2.6% hypochlorite for 5 minutes.
[2] 2.6% hypochlorite for 1 hour.

ence between control and digested lots is of the same remarkable magnitude. The seeds in the digested lots actually performed better than the ones which had their coats removed by hypochlorite solution. Table 2 reveals, furthermore, that seedlings emerged much earlier in the digested lots. Prolonged soaking in water did not improve germination. A trend toward lower germination percentages in lots that stayed for longer periods in the tortoise gut is suggested by the data, but the number of seeds is too small to establish this point.

A third test was conducted in August, 1960, utilizing LA166, the accession with greatest seed dormancy. For this experiment ripe fruits produced by plants grown in the greenhouse at Davis were fed to the tortoises in an effort to simulate the natural situation. A total of 487 fruits were fed to the two animals over a five-day period. Carmine was fed with the tomatoes on the first day. Both animals seemed rather indifferent to the tomatoes, possibly reflecting unfamiliarity, and it was necessary to mix the fruits with mashed bananas in order to achieve ingestion of the entire number. A few fruits were lost in the feeding process. By extracting and counting seeds from 86 fruits of the same batch it was estimated that about 17,400 seeds had been fed to the tortoises in this test.

Seeds were again successfully recovered from the droppings (table 3). In addition, it was possible to count the fruit pericarps, which were very little affected by digestion. The mildness of tortoise digestion is also reflected in the recovery of some 10% of the fruits in an essentially intact condition; such fruits were often broken at the stem end, but seeds and other contents were preserved in normal position. Seeds and carmine first appeared twelve days after the start of feeding. The dye could be detected

TABLE 3. *Seed recovery data for third tortoise feeding test*

Days after feeding	Number of feces	Carmine in feces	Number fruit skins: Empty	Intact	Number recovered seeds: Free	In fruits	Total
8	5	None					
9	2	None					
10	1	None					
11	1	None					
12	2	Strong	27	1	642	11	653
13	3	None					
14	3	Strong	17		338		338
15	2	Moderate	18	4	331	78	409
16	3	Trace	119	16	1,350	290	1,640
17	3	None	56	5	611	105	716
18	3	None	16	3	259	71	330
		Total	253	29	3,531	555	4,086

Approximately 17,400 seeds of LA166 fed to tortoises, starting on July 28, 1960.

TABLE 4. *Germination of seeds recovered in third tortoise feeding test and those of hypochlorite treatment and control*

Treatment	Total planted	Number of seeds: Days after planting 3	4	5	6	7	8	9	10	11	12	13	14	15	16	17–20	Germinated: Number	%
Control	100																0	0
Hypochlorite[1]	100	11	15	13	3	1					1			1	7		52	52
12 days in tortoise free seeds	100		5	2	6	5	1										19	19
15 days in tortoise free seeds	100				2												2	2
15 days in tortoise seeds from intact fruits	78					1											1	1.3
18 days in tortoise	100																0	0

[1] 2.6% hypochlorite for 1 hour.

until the 16th day and seeds were still appearing on the 20th day, the last day of collections. The absence of seeds on the 13th day and other irregularities, we suspect, reflects a slower passage through the smaller tortoise, whose feces alone were collected on that date. A total of 4,086, or 23.5% of the ingested seeds, was recovered.

Germination tests were made without sterilization treatment and included the usual control and lot with seed coats removed. The response was slower and very much smaller than with LA317 (table 4). The best germination—52%—was recorded for the hypochlorite treatment. Next highest was 19% found in free seeds recovered 12 days after feeding. Germination in later recoveries dropped to very low levels. Thus, although digestion did not induce high germination percentages, it permitted *some* germination—a response that was never obtained in controls of LA166. The decrease in germination of the later recovered lots, together with a similar trend observed in the second test, suggest that prolonged retention in the tortoise digestive tract might have an unfavorable effect on seed viability.

A fourth test was conducted to see what effects, if any, tortoise digestion might have on viability of garden tomato (*L. esculentum*) seeds, which do not require special aids for germination at any time after harvest. Several workers have reported (see Discussion) that digestion by domestic animals tends to lower the germination percentage of non-dormant seeds. For this purpose approximately 1,200 seeds of a freshly extracted batch of a large-fruited tomato were fed to the larger tortoise of the pair used in these tests; feces were collected, seeds extracted, and germination tested in the same fashion as for the preceding tests. Seeds first appeared in the excrement seven days after feeding and continued until the 17th day—the last day of collecting. A total of 449 seeds, or 42% of the total fed, was recovered. Samples of 50 seeds each were tested for germination from batches that were recovered 7, 10, 12, and 16 days after feeding. Control germination was perfect; the digested lots germinated 82, 70, 84, and 80 per cent respectively, and their seedlings emerged much earlier than those of the controls. The mean germination of all digested lots was 79 ± 3 per cent, revealing a significant reduction below that of the control level. Despite the general reduction, the data do not show any trend toward lower germination in the lots retained in the tortoise gut for longer periods.

DISCUSSION

The literature reveals numerous examples of the effects of animal digestion upon the viability of seeds of various plant species. Such experiments were conducted as early as the 19th century by Kerner (1902) to settle

contemporary disputes about the possible role of animals in dispersing plant seeds. He ascertained that seeds of a few plant species passed undamaged through the pig, but that the seeds of the majority of plant species tested lost their viability after passage through the digestive tracts of several mammals. Finches, gallinaceous birds, and other species with gizzards destroyed most seeds that had been fed to them. With certain other bird species (blackbird, thrushes, European robin) seeds retained good viability; germination of most seeds was generally retarded, but in the case of *Berberis*, *Ribes*, and *Lonicera*—genera prone to seed dormancy—it was accelerated. The damage that may be inflicted upon seeds by bird digestion is well illustrated by Roessler's (1936) experiment, in which she obtained germination of only 7 of 40,025 seeds from various plant species fed to California linnets.

Rumination effects on seed germination have also interested agronomists, particularly in respect to problems of the spread of weeds in pastures. A complete review of the literature will not be attempted here; instead attention will be called to several well-documented experiments. Harman and Keim (1934) tested the percentage and viability of seeds of six weed species recovered in the feces of calves, horses, sheep, hogs, and chickens. No seeds were recovered from the chickens except 1.9% of velvetweed (*Abutilon abutilon*). For the remaining animals a mean of about 20% of the seeds were recovered. Germination percentages of all except *Rumex acetosella* were improved by passage. Seeds of all tested species lost their ability to germinate after being retained in manure for two months. In similar experiments Burton and Andrews (1948) demonstrated that passage through the bovine digestive tract reduced the viability of certain grass and lespedeza seeds, but actually induced an increased germination of Bermuda grass.

A similar phenomenon is appreciated and exploited by the Ecuadorians. One of us (CMR) learned in conversation with haceneros near Quito that seeds of certain edible species of *Carica* and *Passiflora* are difficult to sprout. This problem is customarily circumvented and plants established by feeding the fruits to workers, who are subsequently instructed to defecate in assigned pits in the fields.

Dr. Herbert G. Baker, Director of the Botanical Garden, University of California, Berkeley, recently informed us of some observations that are pertinent to this discussion. With his kind permission we cite briefly his experiences in Ghana with seed germination of the baobab tree (*Adansonia digitata*) and the sausage tree (*Kigelia africana*). Without treatment these seeds are very difficult to germinate, yet he observed that numerous seedlings were becoming established in rocky areas considerably distant from trees of either species. It became evident that these places were favorite roosts of the baboons, and remains of fruits revealed that they were being devoured by these simians. The extremely strong jaws of the baboons are adequate to crush the very hard, indehiscent fruits of these trees, and, without such aid, the seeds would have no opportunity for dispersal. The germination rate of seeds removed from baboon dung found at these sites was much improved.

Various observations and experiments have therefore established that seeds may retain their viability despite animal digestion and that the seeds of a few species actually germinate better as a result of this process. Plant species showing the latter reaction are naturally prone to seed dormancies. Of all examples known to us, the response in LA317 of the Galápagos tomato accessions is the most dramatic, changing from practically nil to as much as 80% as a result of tortoise digestion. In the examples cited from the literature the greatest improvement did not exceed 20% germination. In the latter cases no net gain was realized because seed recovery from the animal feces was low.

Several questions are raised by the discovery of improved germination of Galápa-

gos tomato seeds by tortoise digestion. One of these is the problem of what physiological mechanism in animal digestion is responsible for the improvement. Three mechanisms might be considered: (1) elimination of a substance that chemically inhibits germination; (2) removal of an obstacle to exchange of substances essential for germination; (3) release of a mechanical barrier to emergence of the seedling. The first proposal is rendered unlikely by the slight, though consistent, improvement of emergence wrought by the seed coat excison treatments. Since this method removes only a small fraction of the seed coat, it would be ineffective in counteracting an inhibiting substance. The interpretation offered by the second proposal is consistent with most of the treatments that improve germination: mechanical removal of part of the seed coat, total removal by hypochlorite, and tortoise digestion. Water and oxygen are the two substances ordinarily absorbed by seeds and essential to germination. Of these, water can be ruled out of consideration because moistening seeds of all Galápagos accessions results in rapid imbibition and swelling. Improved gas exchange would more likely be the factor involved here, as it also is in instances of improved germination effected by hypochlorite, which have been reported in the literature. The third proposal is suggested by the phenomenally rapid emergence of seedlings from seeds requiring repeated treatments with hypochlorite. It has been our experience with the latter that each exposure to the chemical, which erodes away the seed coat, is followed within minutes by the rapid emergence of seedlings from a portion of the seeds, no further germination occurring until immediately after the next treatment. A facilitation of chemical interaction, as proposed in the preceding alternative, could scarcely account for such a rapid response. This concept would also explain the low frequency response to mechanical excision. Here the chance removal of a segment of the seed coat covering the radicle of the embryo would account for emergence. Tortoise digestion does not result in any changes of the seed coat that are visible externally, yet might soften critical layers sufficiently to permit seedlings to emerge. Although our data are not sufficiently critical, the bulk of evidence favors the explanation based simply on seed coat mechanics.

If tortoise digestion improves germination by permitting gas exchange or by relieving mechanical confinement, how is this accomplished? It is beyond the scope of this project to test extensively the various processes of tortoise digestion *in vitro*. Such an approach would furthermore be largely empirical because very little is known about digestion of chelonians in general, let alone Galápagos tortoises in particular. It is nonetheless clear from our tests with the latter and with chickens that mechanical abrasion is not likely the critical part of digestion. It is more likely one of the wide variety of enzymatic reactions that are part of gastric and intestinal digestion. Also, the fermentation of carbohydrates that is promoted by the microflora of the colon should not be overlooked.

In this connection it should be mentioned that we have conducted preliminary tests of the effects of standard laboratory peptic and pancreatic enzymes upon germination of seeds of LA317. The tests were too brief and the results insufficiently consistent to merit complete presentation here. They would justify solely the statement that germination was substantially improved only by pancreatic enzyme treatment. The subject obviously deserves thorough investigation.

The tests reported in this paper suggest that the giant tortoises of the Galápagos Islands might play a role in the germination and dispersal of native tomato species. They do not prove the case. A number of points should be considered in relation to this speculation. In the first place it is the only known natural means of breaking the tomato seed dormancy. Other animals of the rather restricted fauna of the archipelago that might be suspected to play a similar role are the land iguana, *Conolophus subscristatus*, and species of Galápagos mockingbird, *Nesomimus*. The former, en-

tirely herbivorous, is known from the lower elevations where native tomatoes frequent. Being a reptile and herbivorous, its digestive system might be similar to that of the tortoises and might act similarly on tomato seeds. The mocker is likewise largely herbivorous and is found in the areas of the tomato populations. As previously revealed, however, it was observed in nature not to display any interest in the wild tomato fruits. The effects of its digestion are unknown, but it might logically be suspected that the period of bird digestion in general is too short to be effective.

Another consideration is the very long period required for passage through the tortoise gut. According to our results with carmine and tomato seeds, from one to three weeks are required, and it follows from the retrieval of seeds continuing up to the 21st day in the third test that they would have likely continued to appear for several more days. It is also reasonable to suspect that in the warmer climate of their native habitat the tortoises are more active and digestion would take place more rapidly, yet it is clear that a period unusually long for vertebrates is involved. The advantages for dispersal of seeds inherent in such long passages are obvious. According to many observers on the islands, the tortoises roam considerably while feeding and mating. Their agency could therefore be exceedingly effective not only in breaking the seed dormancy but also in providing an effective means of distribution.

We might speculate even further about the distributional role of the tortoises. As just mentioned, they could be highly effective in spreading the native tomatoes within islands. It is also within the realm of possibility that they could play a limited role in the spread from island to island. The problem of such migrations has been treated by several authorities. The Galápagos tortoises will naturally float in seawater, and, with the coincidence of favorable currents, they might rarely drift from one island to another. The distances are short and the viability of the tortoises—for example, their ability to survive long fasts—is known to be enormous. Van Denburgh (1914) discounts the possibility of any such migrations on the basis of the helplessness of the tortoises in water, the risk of injury on treacherous reefs and rocky shores, and the extreme endemism of the island populations. Beebe (1924), on the other hand, who captured a tortoise on Duncan Island and found it adept at swimming in seawater, considers migrations between islands possible.

The distribution of tomato biotypes discloses a very effective means of dispersal. The most abundant type is *L. esculentum* var. *minor*, represented in our tests by LA317. It occurs on at least eight of the major islands and in widely separated loci on each of the two larger islands, James and Albemarle (Rick, 1956). The phenotype of var. *minor* is remarkably stable throughout this range as judged from herbarium specimens and from living cultures. Such a distribution stands in marked contrast to the extreme endemism exhibited by the genus *Scalesia*, for which Howell (1941) lists 19 species native only to the Galápagos and of which only two species occur on more than one island. Such differences in distribution must logically reflect marked differences in effectiveness of dispersal. The interrelationship with the tortoises would provide a very simple and effective means of explaining the widespread distribution of the tomato biotypes.

The possibility of other native animals assisting in the germination and distribution of Galápagos tomatoes has already been discussed. The advent of man on the Galápagos has unfortunately resulted in severe depletion and even extinction of the giant tortoises from some of the islands. Since some of these very islands (Bartholomew, Seymour) still have native tomato populations, obviously some other agent must be responsible for their presence. The land iguana and mockingbird have been previously suggested as other agents. The herds of feral animals, particularly goats, that exist on several islands might also be suspected. Their digestion bears resemblances

to that of the tortoises in respect to their vegetable diet and relatively long digestion period. Examples from the literature have been cited of recovery of weed seeds and improvement of germination of certain species thereof following digestion by domestic ruminants.

The putative relationship between tortoise digestion and tomato seed germination suggests that species which are related to the Galápagos tomatoes and have similar dormancies might also rely to some extent upon animal vectors for germination and dispersal. As revealed by our work, seed dormancies exist, and they can be broken by hypochlorite treatment in some accessions of two other tomato species, and in two related nightshade species. We have no experimental evidence for such animal interrelationships with these species, but in their habitat in western Peru and Chile, a much greater variety of animals exists that might play such a role.

This relationship might have a much wider significance among species of higher plants. Heretofore the adaptive significance of seed dormancies has been explained in various ways. Some dormancies are known that undoubtedly prevent germination under conditions that would be disadvantageous to the growth of the particular species. Others promote survival by preventing immediate germination of all of the seeds. But the effects of animal digestion on germination of a wide variety of higher plant species suggest that the dormancies might have evolved as a special device to promote dispersal. The dormancy devices among these examples are different; to wit, in the Galápagos tomatoes it appears likely to be a mechanical obstruction to germination; impermeability of the seed coat is the factor in at least some of the species tested by Harman and Keim (1934); the after-ripening phenomenon is characteristic of the genera that showed improved germination in Kerner's (1902) tests; the processes concerned with other examples may still be unknown. Animal digestion is therefore effective in breaking seed dormancies of several different types. Thus the various seed dormancies might have evolved not only to prevent germination under unfavorable conditions but also to facilitate dispersal by animal agents.

Finally, the origin of such relationships between animal digestion and seed germination presents a serious problem. Passage through the animal gut can be readily visualized as an effective means of dispersal, but its role in germination is another matter. According to all evidence, seeds of these native tomatoes will not germinate unless exposed to such treatment. The tomatoes would then presumably be entirely dependent upon the animal vector for completion of their life cycle. Although such a relationship is completely effective in guaranteeing a dispersal commensurate with the wanderings of the responsible animal, the fate of the plant hinges entirely on the vagaries of the animal. The fate of the forms which mutated from a free-germinating to the dormant-seeded condition would seem to have been even more precarious. If indeed this happened, the premium gained in dispersal must have outweighed the risks involved in developing the dormant condition. This problem is certainly no more serious than the one posed by the origin of the interdependence of *Yucca* species and species of the *Tegiticula* (*Pronuba*) moths that pollinate them.

Summary

Seeds of tomato species native to the Galápagos Islands are subject to extreme seed dormancy of indefinite duration. The dormancy is alleviated by treating the seeds with a strong solution of sodium hypochlorite, which erodes the seed coat. In a search for natural dormancy-breaking devices, the following treatments proved ineffective: soaking in stagnant or well-aerated water, various temperatures applied to wet or dry seeds, application of acids, planting in native soil, and passage through the gut of immature chickens. Additionally, seeds collected from rat droppings found under plants in their native habitat did not germinate. On the other hand, digestion by the native giant tortoise results in marked

improvement in speed and percentage of germination, the degree of response varying with the tomato biotype. Passage through the tortoise gut required from one to three weeks and probably longer. In all tests a minimum of one-quarter of the ingested seeds was recovered, and the seeds revealed no macroscopic effects of digestion. The dormancy mechanism most likely responsible is one of simple mechanical obstruction of emergence by the seed coat. These results suggest that the giant tortoise might be an important natural agent not only in breaking the dormancy but also in effectively dispersing the tomato seeds. Following tortoise digestion, seeds of the garden tomato, which are not prone to dormancy, germinated but at a lower percentage than that of untreated seeds. The digestive reaction that is most likely responsible for alleviating dormancy, the possible role of other animal agents on the Galápagos Islands, and evolutionary aspects of animal digestion as a promoter of germination and of dispersal of plant seeds are discussed.

Literature Cited

Beebe, W. 1924. Galápagos: world's end. G. P. Putnams's Sons, London. 443 pp.

Benedict, H. M., and Jeanette Robinson. 1946. Studies on the germination of guayule seed. U. S. Dept. Agr. Tech. Bull. **921**, 48 pp.

Burton, G. W., and J. S. Andrews. 1948. Recovery and viability of seeds of certain southern grasses and *Lespedeza* passed through the bovine digestive tract. Jour. Agr. Res., **76**: 95–103.

Harman, G. W., and F. D. Keim. 1934. The percentage and viability of weed seeds recovered in the feces of farm animals and their longevity when buried in manure. Jour. Amer. Soc. Agron., **26**: 762–767.

Howell, J. T. 1941. The genus *Scalesia*. Proc. Calif. Acad. Sci., **22**: 221–271.

Kerner, A. 1902. The natural history of plants. London. Blackie and Son. (pp. 862–866.)

Laude, H. M. 1951. Treatments to improve the emergence and stand of smilo grass. Jour. Range Management, **4**: 88–92.

Rick, C. M. 1956. Genetic and systematic studies on accessions of *Lycopersicon* from the Galápagos Islands. Amer. Jour. Bot., **43**: 687–696.

Roessler, E. S. 1936. Viability of weed seeds after ingestion by California linnets. Condor, **38**: 62–65.

Taylor, C. A. 1949. Some factors affecting germination of celery seed. Plant Phys., **24**: 93–102.

Van Denburgh, J. 1914. The gigantic land tortoises of the Galápagos Archipelago. Proc. Calif. Acad. Sci., **2**: 203–374.

HYBRID SWARMS BETWEEN WILD AND CULTIVATED RICE SPECIES, *ORYZA PERENNIS* AND *O. SATIVA*[1]

HIKO-ICHI OKA[2] AND WEN-TSAI CHANG[3]

Received September 16, 1960

Oryza perennis and *O. sativa* f. *spontanea* are found only in the state of nature, but they are both genetically in close relation with the cultivated rice, *O. sativa*. In view of the occurrence of a continuous array of intergrades between them, we call them simply *perennis* and *spontanea* types (comp. Morishima, Oka, and Chang, 1961). With respect to hybridization of the spontanea type of wild rice with cultivated rice, the authors have recently reported that in the suburb of Raipur, India, plants of this wild type growing as a weed in rice fields or their proximity showed in various respects intermediate characters between typically wild and cultivated forms (Oka and Chang, 1959). They pointed out that introgressive hybridization and adaptation to habitats disturbed by man might bring about such intermediate populations.

Their observations were limited to populations of the *spontanea* type, which are of annual habit. In view of the tendency of *perennis* populations to store up genetic variability (as shown by Morishima, Oka, and Chang, 1961), hybridization of this wild type with cultivated rice seems to bring about hybrid swarms. This paper deals with such hybrid swarms, found in India, Thailand, and Formosa. It was found that a hybrid swarm could preserve a great amount of variability which, if released, might cover almost the whole range of variations from wild to cultivated types.

MATERIALS AND METHODS

The following populations were observed in this study. Their habitats are illustrated by sketch maps in fig. 1. From each of them, seeds were taken at random on an individual plant basis, and plants raised from the seeds were investigated in Taichung, Formosa.

1. *Control populations*.—A wild population of *perennis* type, W207, and another of *spontanea* type, W203, were chosen as controls. The former was found in a water reservoir, 1 to 1.5 meters deep, close to the campus of the Central Rice Research Institute, Cuttack, Orissa, India. The pond was surrounded by guava orchards and forests. Seeds were collected by the senior author in middle November, 1957, from plants standing in deep water. The *spontanea* type was found in a roadside marsh about 2 km S of the *perennis* site. The habitat was surrounded by fields of upland crops and was already nearly dry when seeds were collected in late October, 1957.

2. *Patna population* (W213-4).—This was a mixture of wild and cultivated plants in a paddy field, found at Patna village, near the site at which W203 was found. The paddy field, about 15 m × 80 m, was situated between a road and a canal with embankment and was swampy. The field belonged to a farmer living nearby, and the rice plants had been broadcast by him at the beginning of the rainy season. Seeds were collected by the senior author in middle November, 1957, a little before maturity.

3. *Sampatoon population* (W218).—This was a wild population seemingly of intermediate *perennis-spontanea* type found in a small meandering stream, about 15 km SW of Chiengmai City, Thailand, along the road to Sampatoon. The stream was about 5 m wide, and on both sides were paddy fields in which glutinous rice was grown; in

[1] Contribution from National Institute of Genetics, Japan, No. 334.

[2] National Institute of Genetics, Misima, Japan.

[3] Taiwan Provincial College of Agriculture, Taichung, Formosa.

[2-3] The writers wish to express their sincere thanks to The Rockefeller Foundation for the financial support (RF 57080; Studies on the origin of cultivated rice) which enabled them to make this investigation.

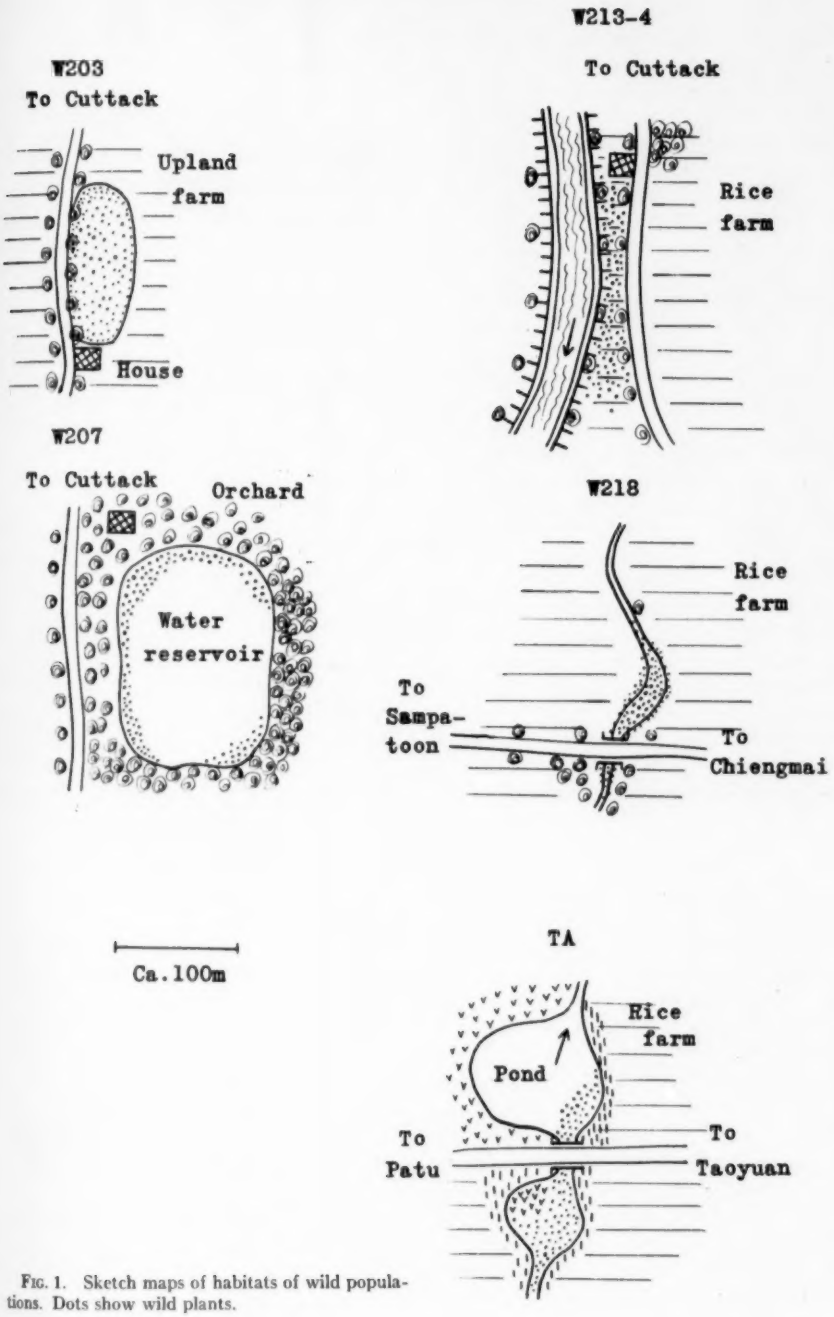


FIG. 1. Sketch maps of habitats of wild populations. Dots show wild plants.

the northern and northeastern parts of Thailand, glutinous rice is grown in almost all paddy farms as the principal food crop. When the senior author visited this spot in late November, 1958, the stream was nearly dry, and the wild plants as well as the cultivated ones in adjacent rice farms were at maturity.

4. *Taiwan populations* (TA, TB, and TC).—The senior author once assumed that the wild rice found in Patu village, Tao-Yuan Prefecture, Formosa, might be *O. sativa* f. *spontanea* (Oka, 1956), but the presence of rhizomes indicates that it is rather *O. perennis*. Three small populations called by the authors A, B, and C, 1 to 2 km apart from one another, are found each in a natural stream about one meter deep. On both sides of the streams are rice farms, bordered by bamboo windbreaks. No other wild population than these three was found in Formosa. Seeds were collected by the junior author in early October, 1957.

Plants from the seeds of these populations were investigated in plant-to-row experiments in the experimental field of Taiwan Provincial College of Agriculture, Taichung, Formosa (24° N). The experiments were conducted as follows: Seeds were water-soaked for germination on June 5, 1958 (1959 for *Sampatoon* population), and seedlings raised in pots were transplanted to the experimental field on June 20. A line which represented a plant of the original population consisted of two to five plants. A population consisted of 40 to 100 lines. Their heading dates ranged from early September (some plants of *Taiwan* populations) to middle November (plants of *perennis* type from India and Thailand). Measurements were taken for each of the plants regarding heading date, ligule length, panicle length, rachis number per panicle, awn length, apiculus hair length, length and width of spikelets, weight of 100 grains, grain shedding, and other morphological traits. In addition, germination tests were made with a part of the plants for examining dormancy. Methods for measuring shedding, dormancy, and other characters, as well as temperatures and day-lengths at Taichung, are given by Morishima, Oka, and Chang (1961).

Results of Observations

Control *Populations and Discriminant Formulas*

It was pointed out by the present authors that the principal directions of differentiation in Asian wild rice populations could be represented by two component axes, one representing the differences in the character complexes of *perennis* and *spontanea* types, and the other those of wild and cultivated types (Morishima, Oka, and Chang, 1961). In accordance with this view, two discriminant functions, one for classifying *perennis* and *spontanea* types, and the other for classifying wild and cultivated types, were constructed as a tool for a synthetic estimation of variations in various characters. The former discriminant function (X_1) was computed from data for 23 wild populations (10 of *perennis* and 13 of *spontanea* type) grown together with the materials of the present study. The characters used were ligule length (L), panicle length (P), rachis number per panicle (R), and weight of 100 grains (W). The latter function (X_2) for classifying wild and cultivated types was computed from the data for the above 23 wild populations and 34 cultivated varieties from various Asian countries, which were also grown simultaneously. The characters used were rachis number per panicle (R), weight of 100 grains (W), and spikelet width (B). The formulas obtained were

$$X_1 = 1.6\,L_{(\mathrm{cm})} - P_{(\mathrm{cm})} - 0.9\,R + 12.4\,W_{(\mathrm{gm})}, \text{ and}$$

$$X_2 = R + 3\,W_{(\mathrm{gm})} + 4\,B_{(\mathrm{mm})}.$$

Offspring of the *Control* populations were then scattered by using these two discriminant formulas, as shown in fig. 2. In the figure, three cross configurations are also given, which show means of many *perennis* and *spontanea* populations and cultivated varieties by their intersection points, and twice the standard deviations for intrapop-

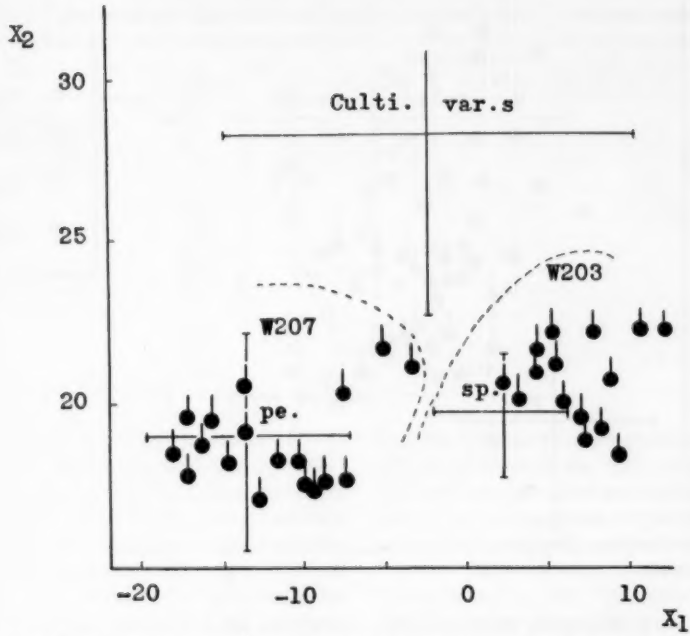


FIG. 2. Seed-raised plants of *Control* populations scattered by scores of two discriminant formulas, one (abscissa) classifying *perennis* and *spontanea* types, and the other (ordinate) classifying wild and cultivated types. Cross configurations:

Culti. Mean ± 2σ for cultivated varieties from various Asian countries. σ represents inter-varietal variation.

pe. Mean ± 2σ for populations of *perennis* type. σ represents mean intrapopulational variation.

sp. Mean ± 2σ for population of *spontanea* type. σ represents mean intrapopulational variation.

ulational (for wild populations) or intervarietal (for cultivated varieties) variations by the length of the lines. It is recognized in fig. 2 that typical populations of the *perennis* and the *spontanea* type are distributed each in a certain area. It is also found that *perennis* populations have greater intrapopulational variability than *spontanea* populations, as pointed out by Morishima, Oka, and Chang (1961).

Patna *Population; a Hybrid Swarm in a Paddy Field*

Plants of wild type in this population could be roughly distinguished from cultivated ones by their awnedness. With the view to examining if this population is a mixture of wild and cultivated plants or a hybrid population, awnedness *vs.* awnlessness, shedding (grains completely or partly shed at maturity) *vs.* non-shedding, and variations in several other characters were compared, using the offspring of the plants grown in Taichung. The results proved that all these characters were recombining. The population may then be said to be a hybrid swarm between wild and cultivated plants.

Plants of this population were then conventionally divided into the following four classes: (1) plants of pronounced cultivated type in all characters, (2) plants with cultivated traits except shedding, (3) plants with wild traits except non-shedding, and (4) plants of wild type in all characters. The

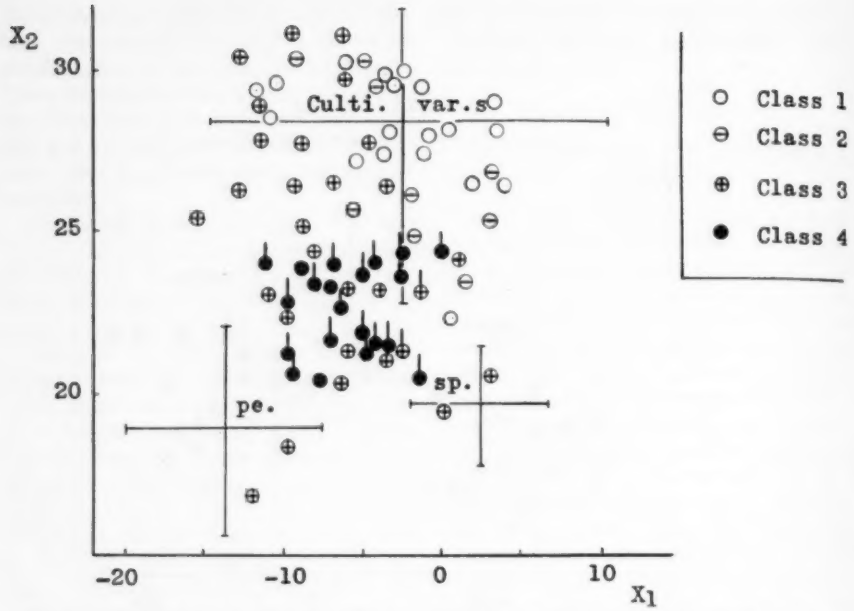


FIG. 3. Scatter diagram for *Patna* population. The abscissa and ordinate axes represent scores given by the two discriminant formulas used in fig. 2.

plants of Class 3, showing many wild and a few cultivated characters, were markedly more heterozygous than those of other classes.

The same conclusion was also drawn from the scatter diagram in fig. 3, in which the offspring of this population were scattered by the two discriminant functions presented in the previous chapter, one for *perennis vs. spontanea* types, and the other for wild *vs.* cultivated types. In the diagram, the plants of Class 4 are distributed between the areas of *perennis* and *spontanea* populations, more or less approaching the cultivated type, and those of Classes 1 and 2 are in the area of cultivated varieties, while those of Class 3 are distributed over the whole area covering *perennis*, *spontanea*, and cultivated types. Thus, the plants of Class 3, which are of wild type in most characters, seem to preserve a large amount of genetic variability.

Pollen fertility and regenerating ability of these four classes are shown in table 1, compared with those of control populations. As the table shows, the *perennis* control population produced a number of partially fertile plants in its offspring and had a high regenerating ability, while the *spontanea* control population had a high degree of fertility and a low regenerating ability, as pointed out by Morishima, Oka, and Chang (1961). The high fertility and relatively low regenerating ability of the plants of Classes 3 and 4 indicate that they are propagated mainly by seeds, though they appear to be of *perennis* type.

The *Patna* population was growing in a paddy field, though whether or not cultivation is practiced there every year is uncertain. Plants of wild type may be at a disadvantage in cultivated condition, and may be suppressed by cultivated plants due to their strong competitive ability; according to Narise and Sakai (1960), wild plants have a lower competitive ability than the cultivated ones, if tested in cultivated condition. They may further receive pollen from coexisting cultivated plants, since their outcross-

TABLE 1. *Percentage of regeneration and variation in pollen fertility in different populations*

Population	%of regeneration	% of good pollen												No. of plants
		100	95	90	85	80	75	70	60	50	40	30	20	
Control Popul's.														
W207 (*perennis*)	100	20	24	9	2	1	2		1	2	1	1	1	64
W203 (*spontanea*)	3.9	44	5											49
Patna Popul.														
Class 1	0	78	3	2					1					84
Class 2	0	36	5	1										42
Class 3	16.7	19	7			1	1	1						29
Class 4	20.0	64	4	1										69
Sampatoon Popul.	91.0													355
Taiwan Popul's.														
TA	71.7	14	10	2		2								28
TB	78.3	50	19	4	1	2		1	1					78
TC	75.6	30	22	6	3		2	3	8	3				77

ing percentage might be as high as 30% to 40% (Oka, 1956; Oka and Chang, 1959; Sakai and Narise, 1959). It does not seem that they can persist in this paddy field very long, unless new wild plants continually immigrate into the habitat. However, it is possible that they change in the direction of the cultivated type through selection of segregants, and add new deviates to the cultivated population, as will be discussed later. The *Patna* population was the only one which was apparently a hybrid swarm among about sixty populations of wild rice visited by the senior author in India. The relatively rare occurrence also indicates that such a hybrid swarm can exist only temporarily under certain exceptional conditions.

Sampatoon *Population; a Wild Population Containing the Glutinous Gene*

As mentioned above, glutinous rice is grown in almost all paddy fields in the northern and northeastern parts of Thailand; it can be said that so far as the cultivated rice is concerned, a large portion of the land is occupied by the glutinous gene. However, all wild species of *Oryza* seem to be essentially non-glutinous. For instance, the writers have examined with regard to this endosperm character a large number of seeds from more than sixty Indian populations belonging to *O. perennis*, *O. sativa* f. *spontanea*, and other species. No single glutinous grain was found among them. Therefore, the frequency of the glutinous gene in wild populations in the "glutinous area" of Thailand may be a good material for investigating the introgression of genes of cultivated plants into wild populations.

From this viewpoint, the senior author has examined in the "glutinous area" of Thailand many plants taken from 14 wild populations growing in proximity of paddy fields and 7 cultivated ones. The results of iodine test of their grains are given in table 2. The data in the table show, regarding cultivated plants, that none of the seven glutinous populations was free from non-glutinous plants. It is known that the glutinous gene is at a selective disadvantage because of certation in a heterozygote, and due to a relatively low productivity of glutinous homozygotes. This might be the reason for the wide spread of non-glutinous gene in glutinous cultivated populations. The percentages of outcrossing given in the table were estimated on the basis of the number of non-glutinous grains in glutinous homozygotes and the frequency of non-glutinous gene in the population. They are within the range of values so far reported for cultivated rice.

It is evident in table 2 that most wild populations contained the glutinous gene at a low frequency, but in a few it was rather high. This indicates that populations of hybrid nature may occur only under certain conditions. Of the two populations show-

TABLE 2. *Frequency of the glutinous gene in* perennis, intermediate, spontanea, *and cultivated populations in northern and northeastern parts of Thailand*

Population number	Type[1]	Locality (Prefecture)	No. of plants			Freq. of *gl* gene (%)	% of outcrossing
			++	+ *gl*	*gl gl*		
W217	*Perennis*	Chiengmai	73	1	1	2.0	
W219	"	"	59	1	0	0.8	
W225	"	Konkhen	54	0	0	0.0	
W229	"	"	42	0	0	0.0	
W223	"	Nongkai	28	0	0	0.0	
W218	*Intermediate*	Chiengmai	55	42	9	28.3	43.98
W220	"	"	48	6	0	5.6	
W222	"	"	115	2	0	0.9	
W228	"	Konkhen	31	5	0	6.9	
W235	"	Nongkai	11	3	3	26.4	
W226–7	*Spontanea*	Konkhen	67	0	0	0.0	
W230	"	"	18	0	0	0.0	
W236	"	Udorn	28	0	0	0.0	
W240	Cultivated	Chiengmai	13	2	30	68.9	0.21
W241	"	"	6	0	22	78.6	2.06
W242	"	"	2	0	78	97.5	0.00
W243	"	Konkhen	4	0	20	83.3	3.58
W244	"	"	4	1	7	62.5	3.41
W246	"	Nongkai	6	0	57	90.5	2.53
W248	"	"	5	0	68	93.2	1.25

[1] Classified by Morishima, Oka, and Chang (1961) based on the results of component analysis of between-character correlations.

ing a high percentage of the glutinous gene, W218 and W235, only the former (*Sampatoon* population) was studied, since of the latter an insufficient number of plants was available.

We may observe the numbers of non-glutinous (++), heterozygous (+ *gl*) and glutinous (*gl gl*) plants first. According to Nei (1953), when selection does not act, the frequency of three genotypes, *AA*, *Aa*, and *aa*, in a population is determined by the percentage of outcrossing (λ) and the relative frequency of genes (x:1−x) as follows:

$$f(AA) = \frac{(1-\lambda)x + 2\lambda x^2}{1+\lambda};$$

$$f(Aa) = \frac{4\lambda x(1-x)}{1+\lambda};$$

$$f(aa) = \frac{(1-\lambda)(1-x) + 2\lambda(1-x)^2}{1+\lambda}.$$

From the data in table 2, the frequency of non-glutinous gene (x) in the *Sampatoon* population was found to be 0.717. The frequency of outcrossing was estimated to be 0.4398, from the frequency of non-glutinous grains in presumably glutinous homozygotes. When these values are used in the above formulas, the following frequencies of ++, + *gl*, and *gl gl* plants are expected.

	++	+ *gl*	*gl gl*	Total
Expected frequency	0.593	0.248	0.159	1.0
Expected number	62.8	26.3	16.9	106.0
Observed number	55	42	9	106
Chi-squares	0.97	9.37	3.69	14.03 ($P<0.01$)

The above figures show that the number of heterozygotes was larger, and that of glutinous homozygotes smaller, than expected. This indicates that heterozygotes were at a selective advantage, and glutinous homozygotes were at a disadvantage in this population.

As an example to show the relative heterozygosity of ++, + *gl*, and *gl gl* plants, the genetic components of variances of spikelet length, measured from the Taichung material, are shown in table 3. The data in the table show that the three classes did not considerably differ from one another in the

TABLE 3. *Genetic components of variances of spikelet size in different populations*

Population	Spikelet length (mm)			Spikelet width (mm)		
	Mean	σ_p^2	σ_o^2	Mean	σ_p^2	σ_o^2
Control Popul's.						
W207 (*perennis*)	7.99	.0490	.0756	2.17	.0060	.0148
W203 (*spontanea*)	8.40	.0172	.0155	2.77	.0094	.0013
Sampatoon Popul.	8.37	.0559	.0739			
++ plants	8.43	.0567	.0680			
+ *gl* plants	8.30	.0499	.0906			
gl gl plants	8.47	.0488	.0059			
Taiwan Popul's.						
TA	8.24	.0197	.0733	2.83	.0136	.0743
TB	8.47	.0410	.1660	2.85	.0051	.0380
TC	7.83	.0012	.1343	2.95	.0007	.0234

σ_p^2 ... Genetic component of variance between lines.
σ_o^2 ... Genetic component of variance within line.

variance among lines σ_p^2, but differed markedly in the variance within lines σ_o^2. The *gl gl* plants were found to be highly homozygous as compared with the + *gl* ones. It seems that they are mostly due to self-pollination of + *gl* plants, and are at a disadvantage on account of their homozygosity for genes of cultivated rice.

In order to examine how the ++, + *gl*, and *gl gl* plants carry other genes of cultivated rice, a discriminant formula which maximized the difference between ++

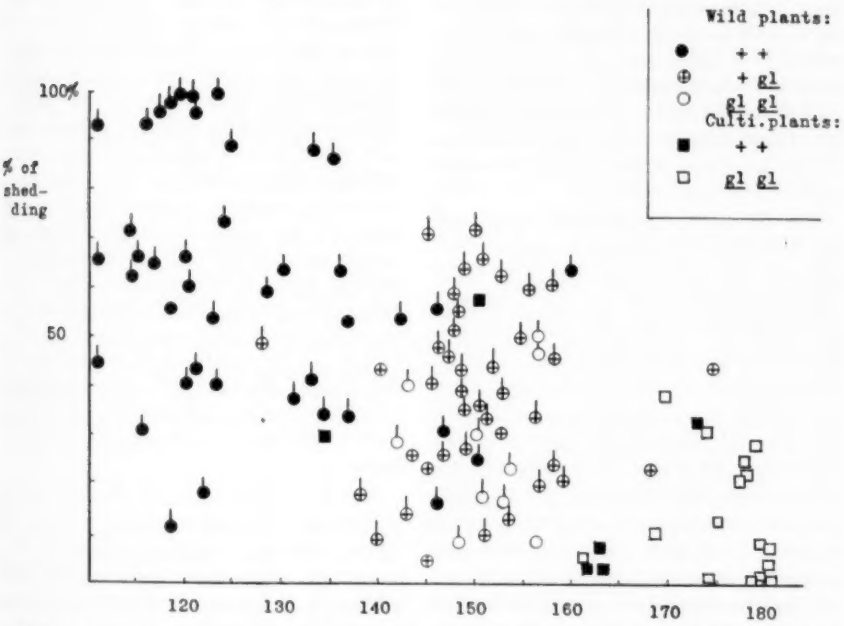


FIG. 4. Plants of *Sampatoon* population scattered by the score of a discriminant formula for classifying non-glutinous wild plants and glutinous cultivated ones growing nearby (abscissa) and the percentage of shedding (ordinate). Offspring line means are shown.

TABLE 4. *Comparison between the three* Taiwan *populations and their offspring regarding variations in various characters*

Popul.	Generation	Phenol reaction		Apiculus color		Spikelet width (mm)							Awn length (cm)								
		+	−	Colored	Colorless	2.2	2.4	2.6	2.8	3.0	3.2	3.4	0	1	2	3	4	5	6	7	8
TA	Original	33	0	30	3		5	4	2	1					1	6	8	12	6		
	Offspring	72	4	62	32	1		3	5	6	5	1	2	1	1	2	2	12	6	1	
TB	Original	57	0	56	1			4	7	5						2	9	21	19	5	1
	Offspring	169	2	149	36			3	9	10	3	2	6		3	8	8	18	23	8	5
TC	Original	59	0	57	2		5	14	2					6	1	2	9	18	14	9	
	Offspring	72	39	81	45			1	2	6	4		25	4	5	12	14	10	4	1	

plants of the *Sampatoon* population and cultivated plants in the adjacent rice fields was constructed by combining measurements of spikelet number per panicle, panicle length, and spikelet width. Using the score given by this discriminant formula and the percentage of grain shedding as abscissa and ordinate axes, offspring lines of the *Sampatoon* population and a cultivated population of the adjacent rice field were scattered in a diagram. The result is shown in fig. 4. It is found in the figure that + *gl* as well as *gl gl* plants are located between the areas of ++ wild plants and cultivated ones. Like those of Class 3 of the *Patna* population, plants with glutinous gene of this population seem to be heterozygous for many traits of cultivated rice.

The difference between the *Patna* and the *Sampatoon* populations is that the former was in a paddy field, while the latter was in a state of nature. However, the habitat of the latter may have been also disturbed by man, since the stream in which the population grows is utilized for irrigation and there are rice farms on both sides. Also the wild plants in this population may continually get pollen from adjacent rice fields. As the high frequency of + *gl* plants indicates, some hybrid plants may keep pace in this habitat with truly wild ones, but plants homozygous for genes of cultivated rice may be eliminated. A certain level of heterozygosity may be kept in the population if migration and selection counteract each other, bringing about an equilibrium.

Taiwan *Populations; Wild Populations Heterozygous for Genes of Cultivated Rice*

As mentioned above, the three small populations of *perennis* type found in Patu village are the only known wild rice in Formosa. The plants in the natural habitats seem to be completely of wild type, but their offspring segregate into various types. For instance, all the plants in natural populations showed positive phenol reaction of glumes (tested by 1.5% phenol solution), and most showed anthocyanic pigmentation at the apiculus. However, as shown in table 4, many plants with negative phenol reaction, as well as with colorless apiculus, were produced in their offspring. Phenol reaction, positive or negative, is determined by one gene, *Ph*:*ph*. Apiculus pigmentation is known to be controlled by three complementary genes, *C*, *Sp*, and *A* (Nagao and Takahashi, 1956), while colorless plants are in most cases of the genotype *c Sp A* or *C sp A*. In view of the high frequencies in the offspring of plants with recessive phenotypes, the plants in the natural populations seem to be mostly heterozygous for these genes.

Another evidence for the heterozygosity of plants of *Taiwan* populations is found in table 3, regarding the values of genetic components of variances of spikelet length and width. It is recognized in the table that within-line variances (σ_o^2) are markedly larger than between-line variances (σ_p^2) in *Taiwan* populations.

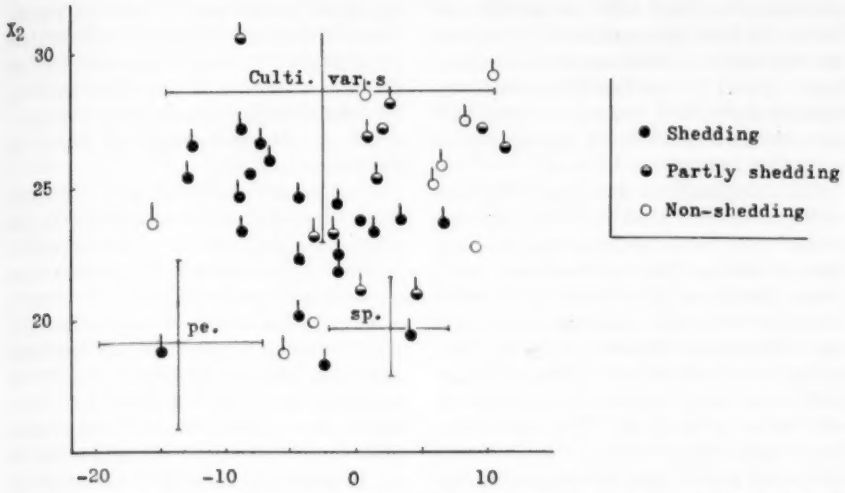


FIG. 5. Scatter diagram showing that seed-raised plants of *Taiwan* populations are distributed in a sphere from *perennis* and *spontanea* to cultivated types. The abscissa and ordinate axes represent scores given by the two discriminant formulas used in fig. 2.

A comparison between the plants in natural populations and their offspring was made further regarding awn length and spikelet width. The results are given in table 4. As the data in the table show, the wild plants were mostly awned and had relatively narrow spikelets, but their offspring varied in a wide range. Colorless apiculus, awnlessness, and broad spikelets are characteristics of cultivated plants as compared with wild ones. Further, a part of the segregants showed non-shedding, rapid overcoming of dormancy, and other characters of cultivated plants. These facts indicate that the plants of *Taiwan* populations are mostly heterozygous for genes of cultivated plants. Among the three populations, TC seems to be the most heterozygous one.

Offspring of the three populations, 10 to 15 from each, were scattered by the two discriminant functions presented above, X_1 for *perennis vs. spontanea* types and X_2 for wild *vs.* cultivated types. As shown in fig. 5, the offspring varied from *perennis* to *spontanea* as well as to cultivated types, in the same manner as the plants of Class 3 of the *Patna* population.

In order to estimate the percentage of outcrossing, several wild plants of *Taiwan* populations which were ascertained to be homozygous for non-glutinous gene, were planted in the center of eight surrounding plants of a glutinous cultivated variety, and seeds were gathered from the panicles of the wild plants which flowered at the same time as the surrounding ones. The frequency of outcrossing, thus found as the frequency of glutinous heterozygotes in the offspring, was 35/114 or 30.7%. This value is comparable with that found in *Sampatoon* population, as well as those estimated by statistical methods in natural populations of *perennis* and *spontanea* type (Oka and Chang, 1959; Sakai and Narise, 1959). Assuming the frequency of outcrossing in *Taiwan* populations to be 30.7%, the frequency of heterozygotes cannot be higher than 23.5% according to the formula of Nei (1953). Actually, it may be nearly 100% as mentioned already. This strongly suggests that homozygotes are eliminated in the natural populations.

The frequencies of partially fertile and of regenerating plants in the offspring of these three populations are given in table 1. The

abundance of plants with low fertilities as well as the high regenerating ability suggest that the natural populations are of perennial habit. It may be said that the *Taiwan* populations are hybrid swarms between wild and cultivated forms which are maintained asexually.

The habitats of the three populations are each limited to a small patch in a stream, about 5 m × 30 m, and the number of plants seems to be less than one thousand. Some twenty-five years ago, another population was found in a water reservoir in a village near Hsianghsan, Hsinchu Prefecture, but it disappeared around 1950. Thus, the three populations found at present may be considered to be exceptional. They are highly heterozygous for genes of cultivated rice as mentioned above, and are exposed to the flow of genes from adjacent rice fields. They may tend toward extinction because of the disadvantageous effects of genes of cultivated rice. It seems that they are maintained by rhizomes in usual years, but in a year of drought the plants markedly decrease and new plants come up from seeds. In this case, it may be supposed that genes from cultivated plants are linked with others of wild plants in such a manner as $(A-b)/(a-B)$, and homozygotes due to self-pollination are mostly eliminated, leaving only heterozygotes in the population. It is possible that if a wild population absorbs too many genes of cultivated plants, the genes may work like balanced lethals due to linkages, insofar as the population remains in wild condition.

Discussion

A great amount of genetic variation will be required for the evolutionary change from a wild plant to a cultivated one. As the sources of genetic variability in a population, we know mutation and hybridization; the latter might be more frequent than the former, and might blend genetic variations in a form suitable to be tested by selection. As pointed out by Anderson and Stebbins (1954) with many examples, "accelerated introgression in disturbed habitats" may rapidly create new deviates which are adaptive to various new habitats, if such habitats are available. It may be assumed that, in the same manner as in our plant breeding, hybridization might have been the main procedure of natural breeding to create the cultivated rice.

Though cultivated rice is a self-pollinated plant (percentages of outcrossing so far reported ranging from 0% to 6.8%), the wild plants of both *perennis* and *spontanea* types are cross-pollinated at about 30% to 40%, as already mentioned. Hybridization between wild and cultivated rice has been repeatedly observed in India (Roy, 1921; Bhalerao, 1928; Mitra and Ganguli, 1932; Hector, 1935; Sampath and Govindaswami, 1958; etc.). Thus, wild rice plants may be continually cross-pollinated not only by plants of the same population but also contiguous populations. On account of the difference in the outcrossing percentage, the gene flow from cultivated to wild plants may be more vigorous than in the opposite direction. Accordingly, if a population with many genes of cultivated type was once established, it would share its genes with contiguous wild populations.

The present authors have investigated introgressive hybridization between wild populations of *spontanea* type and cultivated plants (Oka and Chang, 1959). They showed that the populations growing as a weed in rice fields or their proximity, whose habitats are disturbed by man, were of intermediate type between true *spontanea* and cultivated plants, and they assumed that the genotype of a population of hybrid origin would approach that of the cultivated form in accordance with the "cultivation pressure," or the effect of disturbance of habitat by man which works as such a pressure.

Along this way of thinking, it seems that if a given population is propagated only by seeds, hybrid derivatives are relatively rapidly tested by selection and populations approaching a cultivated type may be established only when niches under a cultivation pressure are simultaneously provided for

the new genotypes. In contrast, if the plants are partially asexually propagated, the F_1 and back-cross derivatives, which might be phenotypically of wild type on account of the recessiveness of genes of cultivated rice (as shown by Mitra and Gangula, 1932; Kadam, 1933; the writers' unpublished data; etc.), may be retained in the population, and a hybrid swarm may result.

The populations investigated in this study, whose constituent plants were of *perennis* or intermediate *perennis-spontanea* types, may be said to be hybrid swarms between wild and cultivated plants. In all of them, the plants preserve in heterozygotic condition a large amount of genetic variability, which, if released, might cover almost the whole range of variations from *perennis* to *spontanea* as well as to cultivated types. It seems that such a potentiality of *perennis* populations to accumulate genes of cultivated rice may be of great significance in the creative process of cultivated forms from wild rice. This also supports the authors' view that wild rice of the *perennis* type is more likely the progenitor of the cultivated forms than is the *spontanea* type.

A hybrid swarm may occur when a wild population grows in contact with cultivated plants, but its genotypic constitution may be rather unstable. It may be assumed that an agent influencing gene frequency in such a hybrid swarm might be the difference in the outcrossing percentage between plants of the wild and the cultivated type, because plants of the latter having a low outcrossing percentage will pollinate those with higher percentage, but will not be pollinated by the others. Then, in a closed population, cultivated types would increase unless eliminated by selection. According to a mathematical computation by Mr. K. Hinata of National Genetics Institute, Japan (unpublished), an equilibrium of gene frequency can be established if the difference in outcrossing percentage and the counteracting selection are balanced, while a slight deviation from the balance will result in fixation or loss of the relevant genes. Actually, the outcrossing percentage as well as the selection coefficient might depend on the environment. Thus, the existence of hybrid swarms between wild and cultivated plants may be rather transitory.

A hybrid swarm may, however, contribute new variants to the cultivated rice, or may produce forms which can co-exist with cultivated plants as weeds, if its habitat happens to be under cultivation pressure, or if plants released from it are distributed in cultivated niches. Various plants of the cultivated type were actually obtained from *Taiwan* populations by the authors. Thus, new cultivated varieties may be continually produced by hybridization. It seems that the creation of cultivated rice is not an event which occurred once, but is in progress even at the present time in certain localities in tropical countries. Breeding experiments of hybrids between wild and cultivated plants, as well as mathematical investigations of the evolutionary processes, will throw more light on these problems.

Summary

Several wild rice populations supposedly of hybrid origin between wild and cultivated forms have been analyzed. First, *Patna* population, found in the suburb of Cuttack, Orissa, India, though it appeared to be a mixture of wild and cultivated plants growing in a paddy field, was shown to be a hybrid swarm. A part of the plants, having many wild characters and a few cultivated ones, were found to be highly heterozygous for genes of cultivated rice. Secondly, *Sampatoon* population, found in a stream running through a paddy farm of glutinous rice in the suburb of Chiengmai, Thailand, was observed. It consisted of plants of intermediate *perennis-spontanea* type, and contained many heterozygotes for the glutinous gene. The offspring of the heterozygotes tended in various characters to be intermediate between the wild and the cultivated type. It was also found that though in northern and northeastern parts of Thailand glutinous rice is grown in almost all paddy farms, the majority of wild populations growing in proximity of glutinous rice fields

had a low frequency of the glutinous gene, so that hybrid swarms might be regarded to be rather rare. Thirdly, three small populations found in Tao-Yuan Prefecture, Formosa, which were phenotypically of *perennis* type, were observed. They were found to be highly heterozygous for genes of cultivated rice. Thus, it is pointed out in general that a hybrid swarm could preserve a great amount of genetic variability, which might cover the whole range of variation from the wild to the cultivated type. Based on the results of these observations, emphasis is laid on the significance of hybridization in the evolution of cultivated rice.

Literature Cited

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an evolutionary stimulus. Evolution, **8**: 378–388.

Bhalerao, S. G. 1928. The wild rice (*Oryza sativa*) of the Bombay Presidency. Poona Agr. College Mag., **20**: 45–49.

Hector, G. P. 1935. Breeding and genetics of rice in India. Proc. World's Grain Exh. & Conf., Regina, Canada, 1933, **2**: 121–126.

Kadam, B. S. 1933. Genetic analysis of rice. Proc. Ind. Acad. Sci., **4**: 224–229.

Mitra, S. K., and P. M. Ganguli. 1932. Some observation on the characters of wild rice hybrids. Ind. Jour. Agr. Sci., **2**: 271–279.

Morishima, H., H. I. Oka, and W. T. Chang. 1961. Directions of differentiation in populations of wild rice, *Oryza perennis* and *O. sativa* f. *spontanea*. Evolution, **15**: 326–339.

Nagao, S., and M. Takahashi. 1956. Genetical studies on rice plants. XIX. The third gene in apiculus coloration. Jap. J. Bot., **15**: 141–151.

Narise, T., and K. I. Sakai. 1960. Competition experiment with wild rice. Ann. Rep. Nat. Inst. Genetics, Japan, **10**: 66–67.

Nei, M. 1953. Mathematical studies on the breeding behavior of partially allogamous plants. Jap. J. Breed., **3**: 1–5.

Oka, H. I. 1956. Observations on natural populations of Formosan wild rice. Ann. Rep. Nat. Inst. Genetics, Japan, **6**: 46–47.

—— and W. T. Chang. 1959. The impact of cultivation on populations of wild rice, *Oryza sativa* f. *spontanea*. Phyton, **13**: 105–117.

Roy, S. C. 1921. A preliminary classification of the wild rices of the Central Provinces and Berar. Agr. J. India, **16**: 365–380.

Sakai, K. I., and T. Narise. 1959. Studies on the breeding behavior of wild rice (*Oryza rufipogon* and *O. perennis*). Ann. Rep. Nat. Inst. Genetics, Japan, **9**: 64–65.

Sampath, S., and S. Govindaswami. 1958. Wild rices of Orissa—Their relationship to cultivated varieties. Rice News Teller, July 1958 issue.

HISTORICAL ZOOGEOGRAPHY OF AUSTRALIAN MAMMALS

GEORGE GAYLORD SIMPSON
Museum of Comparative Zoology, Harvard University

Received January 30, 1961

The exceptional evolutionary interest of islands has been universally recognized ever since, and indeed even before, general acceptance of the truth of evolution. Darwin and the Galápagos Islands at once rise to mind, along with a host of subsequent studies on, for example, the East Indies, the West Indies, Hawaii, and Madagascar. Nowhere else is the interplay of migration, isolation, and local radiation so clearly displayed. The peculiarities of insular faunas, usually impoverished and unbalanced in comparison with continental faunas, involve special ecological factors. In detail, other special evolutionary phenomena are strikingly displayed, for example the founder principle (Mayr, 1942) and convergence.

Many insular phenomena are rather closely correlated with the sizes of the islands concerned. When the islands approach and reach continental size, those phenomena become more complex and more difficult to interpret, but also richer and on a grander scale. All the continents (even Europe as distinct from Asia) have themselves been islands at one time or another. Only for Australia and South America, however, was strong isolation recent enough and sufficiently long-continued to be reflected by fully developed and extensive insular phenomena in the recent faunas. That is, of course, one of the best known zoogeographic facts, already emphasized by Wallace and familiar in even elementary treatments of zoology ever since.

The case of the mammals of South America, with abundant fossil evidence, has been analyzed both broadly and in considerable detail (e.g., Simpson, 1950, and references there). Equal or perhaps even more attention has been paid to the insularity of the Australian mammalian fauna. There, however, a satisfactory over-all treatment of the whole problem has not recently been attempted. The fossil evidence is exiguous, and most of the historical zoogeographic studies of the recent fauna have been either so general as to lack significant detail or so partial and confined to particular groups and areas as to give an inadequate grasp of the whole. The purpose of the present paper is to supply that need, even though in an incomplete and inconclusive way. The attempt is made to place the data on mammals of the whole Australian Region available in previous publications by others into a tentative picture of the historical zoogeography of Australia. For this purpose the mammalian fauna of Australia proper (with Tasmania) is taken as a unit, and local distributions within the continent, affected as much by ecological as by strictly historical factors, are usually ignored.

GENERAL NATURE OF THE FAUNA

Two of the usual characteristics of insular faunas, impoverishment and endemicity, are evident in the general makeup of the Australian fauna as summarized in table 1. Both factors vary with categorical level. The numbers of species (not given in the table) and of genera are not much smaller than would be expected from the size and environmental ecology of Australia. The number of orders, only five, is, however, even below that of Madagascar, with six. On other continents it ranges from seven[1] in Europe to twelve each in Asia and Africa.

The idea of Australia as the land of marsupials and monotremes has been so much stressed that even some technical studies have overlooked the fact that Australia does have a rich placental fauna. In fact over

[1] The order Primates is not counted in Europe, where it is represented only by man and the highly marginal Gibraltar monkeys, or in Australia. Native Artiodactyla, recently extinct in Madagascar, are counted there but Tubulidentata are not.

TABLE 1. *Australian native recent mammals*

	Families		Genera	
	No.[a]	Endemic[b]	No.[a]	Endemic[b]
Monotremata	2	100%	2	100%
Marsupialia	6	100%	47	100%
Chiroptera	7	0%	21	29%
Rodentia	1	0%	13	77%
Carnivora	1	0%	1	0%
Totals	17	47%	84	67%

[a] The numbers refer to inhabitants of continental Australia and Tasmania (and not the whole Australian Region).

[b] Groups are counted as endemic if confined to the region east of Wallace's line.

half the native orders and families of Australia and nearly half the native genera are placentals. The idea is, however, correct in a way, because the placentals are either of comparatively recent introduction or of only two broad adaptive types (bats and rats). The great majority of ecological niches for land mammals are, indeed, filled by marsupials. The frequent insular characteristic of faunal imbalance is hardly evident in Australia because the ancient, spectacular marsupial radiation has produced an essentially balanced fauna of land mammals within that one order.[2] The broad outlines of placental zoogeography in the Australian Region are nevertheless more complex and in some ways more interesting than for the marsupials.

Endemicity varies not only categorically but also taxonomically. It is complete from specific through subclass levels for monotremes and through superfamily level for marsupials. It is nearly but not quite complete for species of rodents, and near three-quarters for genera, but the single family is not endemic. For bats, specific and generic endemicity is considerable but is decidedly less than for rodents, and none of the seven families is endemic. The one (placental) carnivore is not endemic at any level above the species.

The differences in endemicity reflect the fact that ancestral members of the various orders reached Australia at different times and in different ways, and that rates of evolution have also differed. In these respects each order is a separate case, and they will be separately discussed, with the sequence reversed from that usual in classification.

CARNIVORA

It is well known that the only placental carnivore in Australia at the time of European discovery was the dingo. It interbreeds freely with domesticated dogs and resembles them more than it does any wild species. The general assumption is that dingos are feral descendants of domesticated or semi-domesticated dogs introduced by aboriginal humans, although the archaeological evidence seems to be inadequate or ambiguous. If the assumption is correct, which is probable, this would be a unique case of a population that had passed through a phase of domestication and some degree of artificial selection and then had fully returned to the status of a wild species through many generations in isolation. (That is not really true of, for example, the "wild" horses of the American west.) Such a history may have had unusual genetic and adaptive consequences. Unfortunately it is probably too late to investigate that possibility adequately.

RODENTIA

The numerous species of native rodents in the Australian Region all belong to the family Muridae. They have been studied in most detail by Tate (especially 1951), and with some later changes his data are the main basis for the following discussion. A few Pleistocene or early Recent fossils are known, but as far as yet studied they add virtually nothing of significance. From a historical point of view the recent murids can be divided into four broad groups:

[2] Someone has remarked that if Linnaeus had been an Australian we would classify marsupials as from three to six orders rather than one. It is true that the ordinal poverty of Australia is to some extent an artifact of traditional classification, but the marsupials do seem to be a more compact and more closely related group than any three placental orders covering a comparable ecological range.

I. Local members of the very widespread genus *Rattus*.

II. Old Papuan genera. Nine genera on New Guinea, two of which also occur in Australia.

III. The *Pseudomys* group. Eight genera, almost confined to Australia.

IV. The subfamily Hydromyinae. Nine genera in New Guinea, one in Australia, and one common to both.

These four groups have had different histories, and each is rather complex within itself.

I. *Rattus*. The now ubiquitous species *R. rattus* and *R. norvegicus*, along with *Mus musculus*, were introduced into the Australian Region after the European discovery. Rats of the *R. exulans* group are widely distributed in the Pacific in a way consistent with prehistoric (i.e., pre-European) spread by native canoes and difficult or impossible to explain otherwise. It is in accord with that mode of introduction that the group is well established in New Guinea, which is more in the prehistoric seafaring lanes, and is comparatively rare and marginal in Australia.

Besides those introductions, late and early, there are seventeen species of *Rattus*, nine in Australia, five in New Guinea, and three in both, that are endemic to the region and seem also to be autochthonous in it. Their differentiation there implies considerable antiquity and pre-human immigration of their ancestry. Tate places them in two of his "divisions" and eight of his "groups" of species of *Rattus*, but all could have differentiated from two or less likely from one ancestry migrant through the East Indies. From the degree of differentiation, arrival in New Guinea and hence in Australia could hardly have been much later than early Pleistocene. There has been some limited later Pleistocene or early Recent intermigration between New Guinea and Australia, with *R. ruber* and *gestri* probably moving from Australia to New Guinea and *R. leucopus* probably in the reverse direction.

II. "Old Papuans." *Uromys* and *Melomys*, common to Australia and New Guinea, and *Xenuromys* and *Pogonomelomys*, confined to New Guinea, comprise a group (the "*Uromys* group") of related rats all of which could well have been derived within New Guinea from a single ancestry near or in *Rattus*. Generic differentiation suggests that the ancestry probably reached New Guinea not later than the Pliocene. *Uromys* and *Melomys*, both of which have speciated widely in New Guinea, almost certainly originated there and spread later to Australia. *Melomys* migrated earlier, for it is now widespread in northern and eastern Australia and has developed new species there. *Uromys caudimaculatus*, not specifically distinct from its Papuan relatives and confined to northeastern Australia, must be a comparatively recent migrant. Within this group only lowland and chiefly rain forest forms spread from New Guinea to Australia, and there seems to have been no back migration.

There are five other old Papuan genera endemic in New Guinea: *Mallomys*, *Hyomys*, *Anisomys*, *Pogonomys*, and *Macruromys*. They are not especially related among themselves, aside from all being murids, and seem to have no special relatives elsewhere. They are even more distinct than the *Uromys* group and must be rather old, Pliocene, at least, and probably Miocene, in New Guinea and may represent several different invasions through the East Indies.

III. "Old Australians," or Pseudomyinae. These highly varied but related members of the *Pseudomys* group are the most common and characteristic native rats of Australia proper. They include the following main adaptive types:

1. Medium-sized to small, relatively unspecialized. Most abundant and diverse. *Pseudomys* (including *Thetomys* and *Gyomys*, sometimes given generic rank), *Leggadina*, and *Zyzomys* (including *Laomys*).

2. Large, gregarious to colonial, nest-building, with very large ears. *Leporillus*.

3. Medium large, with short tail and slender feet. *Mastacomys*.

4. Small to moderate in size, with long ears, tail, and feet, saltorial. *Notomys*.

5. Very large, arboreal, with long, hairy tail. *Mesembriomys*.

6. Large to medium in size, eyes large and ears long, hopping, with long hind feet. *Conilurus*.

Among themselves these related forms represent a well-marked adaptive radiation which has produced a variety of animals convergent toward several different groups of rodents and rodentlike animals of other continents: true rats and mice (*Pseudomys* and allies), voles (*Mastacomys*), kangaroo rats or jerboas (*Notomys*), squirrels (*Mesembriomys*), and rabbits (*Conilurus*). In addition, one group (*Leporillus*) is fairly unique, not closely convergent toward any other rodents. Although this is a broad gamut for one small group of genera, it is far from occupying all the ecological niches of rodents on other continents.

In spite of their diversity, all these animals could have been derived from one ancestral immigrant. The degree of divergence, most or all of which seems to have occurred within Australia, demands a remote date for that ancestry, probably no later than Miocene but probably also little or no earlier, as true Muridae are not known anywhere before the Miocene. The ancestor must have been a very primitive murid, perhaps before distinct differentiation of the Murinae. Recognition of this group as a distinct subfamily, Pseudomyinae, seems warranted.

The pseudomyine ancestors were probably the first rodents ever to reach Australia proper. Their adaptive radiation then occurred there in the absence of any placental competitors. It seems possible that they replaced some earlier rodentlike marsupials, and it will be interesting to see whether such marsupials appear in the now unknown Australian Oligocene fauna.

Tate apparently tended to think of early rodent colonizations of Australia and New Guinea as separate phenomena, but all Australian rodents certainly came ancestrally from the East Indies or ultimately the mainland of Asia, and it seems highly probable that all reached Australia by way of New Guinea. The only likely alternative route is from Timor to Australia, and Mayr (1944a) has shown that some birds followed that route. However, there are no non-introduced murids on Timor, and although the East Indies–Timor–Australia route is shorter it involves now, and probably did in the Miocene, stronger barriers to migration of land animals.

It seems probable that the pseudomyine ancestors were, like the "old Papuans," early migrants to and through New Guinea. If so, the lineage either died out in New Guinea after reaching Australia or is just possibly represented by the Papuan endemic genus *Lorentzimys*. Tate believed that *Lorentzimys*, a small mouselike or *Leggadina*-like murid, belongs in the *Pseudomys* group, although the not very detailed resemblance could be convergent. If *Lorentzimys* really is especially related to *Pseudomys* (and *Leggadina*), it is at least as probable that it is a survivor of ancestral Papuan pre-pseudomyines as that it was a migrant from Australia to New Guinea.

A single species of *Conilurus* has spread, certainly in late Pleistocene or Recent times, from northern Australia to southern New Guinea.

IV. Hydromyinae. This peculiar subfamily is characterized by basined cheek teeth and reduction or loss of the posterior molars. Of its eleven adequately established genera, nine are confined to New Guinea: *Leptomys*, *Paraleptomys*, *Pseudohydromys*, *Microhydromys*, *Baiyankamys*, *Parahydromys*, *Crossomys*, *Neohydromys*, and *Mayermys*. Another, *Hydromys*, has a species, *H. chrysogaster*, common to New Guinea and Australia. Although the species is now widespread in Australia, the Australian and New Guinean populations are not clearly subspecifically distinct and must represent a quite recent spread from New Guinea.

Xeromys, a divergently specialized member of the subfamily, is confined to a small area in Queensland, Australia. Tate (1951)

considered it "a localized invader from New Guinea during middle Pleistocene time or earlier." That is possible but improbable. The genus has no special, close relatives in New Guinea, and differentiation from surviving New Guinea hydromyines would probably have taken a longer time than since middle or even early Pleistocene. Its distribution suggests a relict rather than a localized invader, and it is probably an early offshoot of Papuan hydromyine ancestry that reached Australia in the Miocene or Pliocene and became divergently specialized there.

Chrotomys and the probably not distinct *Celaenomys* of the Philippine Islands (Luzon) are usually referred to the Hydromyinae. Raven (1935) believed that the subfamily originated in the Philippines and migrated hence to New Guinea and eventually Australia. Tate (1936) first suggested that the subfamily arose in Asia and migrated separately to the Philippines and through the East Indies to New Guinea and Australia. Later (Tate, 1951), he said that the Philippine forms are "possibly descendants of protohydromyines left along the ancient migration route from Asia to Australia." None of the three suggestions is satisfactory. The Philippines are quite remote from the (usual) Asia–East Indies–New Guinea–Australia route and no (other?) land mammals are known ever to have migrated in either direction between the Philippines and New Guinea. Nothing like the hydromyines now occurs anywhere in Asia or the East Indies.

I think it practically certain that *Chrotomys* is convergent toward the Hydromyinae and is of quite different ancestry within the Muridae. It is sharply distinct, as Tate says, "independently specialized," from ("other") hydromyines. The Philippine *Crunomys*, still less like a hydromyine, is roughly intermediate between *Chrotomys* and true Murinae and suggests a line of independent derivation from the latter. The South American *Ichthyomys* resembles *Hydromys* at least as closely as does *Chrotomys*. This is certainly a case of convergence, and it shows that the degree of resemblance between *Chrotomys* and the Hydromyinae could readily arise by convergence.

Everything points to the conclusion that the Hydromyinae, as such, originated not in Asia, in the Philippines, or along the East Indian island stepping stones but in New Guinea itself. The remote ancestry, necessarily near the base of the Muridae and possibly even in pre-murid cricetids, doubtless was Asiatic and migrated through the East Indies, but hydromyine specialization was Papuan. In New Guinea the group had an adaptive radiation analogous to that of the Pseudomyinae in Australia but different and more limited in ecological scope. About half of the genera, including those apparently most primitive (e.g., *Leptomys*) are terrestrial, some of them rather shrewlike. Adaptation to subaquatic and aquatic habits occurred within the subfamily radiation. That adaptation added other convergences, notably toward muskrats and some other aquatic rodents.

Summary on Muridae. The murids of the Australian Region clearly did not reach there over a land bridge but by island-hopping down the East Indian chain. From some time in the Miocene (perhaps but less probably even in late Oligocene) there was probably continual, intermittent drift of murids down that chain. There must have been marked attenuation, with fewer originally Asiatic lineages represented at increasing distances from the mainland. There was local differentiation on various islands along the route, and such differentiation was passed on, in part, to the next stepping stone and eventually to Australia, where final and in some cases most extensive differentiation occurred. Most and probably all of the Australian stocks passed through New Guinea, with varying degrees of differentiation there before spreading onward to Australia. In some instances that differentiation was less than specific; in others it was subfamilial. Although continual, the migrations may be analyzed into three successive waves:

A. Oldest wave, mainly or wholly Miocene. There was some basic radiation in

New Guinea but this wave of invasions was evidently multiple, including up to seven but probably fewer different stocks already phylogenetically distinct before or while passing along the East Indies. Five of these stocks (the "old Papuans") remain endemic in New Guinea. Another radiated there into the Hydromyinae, one lineage (*Xeromys*) of which reached Australia early and another (within *Hydromys*) late. Another stock, the Pseudomyinae, although probably of old Papuan origin, had its major radiation in Australia. A single late species reached New Guinea from Australia.

B. Intermediate wave, probably Pliocene. This involved the probably single stock that evolved into the *Uromys* group in New Guinea. Spread thence to Australia involved first *Melomys* and later *Uromys*.

C. Late wave, Pleistocene or perhaps in part late Pliocene. This involved perhaps only one but probably two partly differentiated lineages of East Indian *Rattus*. It radiated moderately in New Guinea and quickly spread to Australia where it radiated more extensively at the specific level. In the late Pleistocene to Recent there was some further, but quite limited, interchange both ways between New Guinea and Australia. (Human introductions, both prehistoric and historic, followed.)

Throughout, predominant movement has evidently been from smaller East Indian islands to the great island of New Guinea and then from New Guinea to the still greater continent of Australia. That apparently violates the zoogeographic principle that successful colonization tends to go from larger and more varied to smaller and less varied regions. The principle strictly applies, however, to movement into essentially closed communities. Here the predominant tendency was to move from what were, for these animals, more closed to more open communities. Moreover, the stocks involved, although not precisely in the form in which they arrived, were ultimately derived from the largest and most varied land mass of all. In the broad picture, this is another example of the usual sort of spread from central, major masses to marginal and terminal areas (see Darlington, 1957).

TABLE 2. *Chiroptera of Australia and their extensions beyond Australia proper*

Aus.—Australia and Tasmania. N.G.—New Guinea. Isl.—Other islands (into East Indies). As.—Asiatic mainland or beyond.

	Aus.	N.G.	Isl.	As.
Megachiroptera				
Pteropidae				
Pteropinae	X	X	X	X
Pteropus	X	X	X	X
Dobsonia	X	X		
Nyctimene	X	X	X	
Macroglossinae	X	X	X	X
Macroglossus	X	X	X	X
Syconicteris	X	X	X	
Microchiroptera				
Emballonuridae	X	X	X	X
Taphozous	X	X	X	X
Mormopterus	X	X	X	X
Megadermatidae	X	X	X	X
Macroderma	X			
Rhinolophidae	X	X	X	X
Rhinolophus	X	X	X	X
Hipposideridae	X	X	X	X
Rhinonicteris	X			
Hipposideros	X	X	X	X
Vespertilionidae	X	X	X	X
Nyctophilus	X	X	X	
Eptesicus	X	X	X	X
Pipistrellus	X	X	X	X
Chalinolobus	X	X	X	X
Myotis	X	X	X	X
Scoteinus	X	X	X	X
Phoniscus	X	X	X	X
Miniopterus	X	X	X	X
Kerivoula	X	X	X	X
Molossidae	X	X	X	X
Nyctinomus	X		X	X

CHIROPTERA

Data for the bats of the Australian Region have also been taken mainly from Tate (1946), but again with some emendations from later studies.[3] The families and genera here recognized in Australia and Tasmania are listed in table 2, and data on

[3] Some of the later changes in the literature have consisted merely of giving generic names to some of Tate's species and species groups, without further study. Those changes from Tate's arrangement have not been justified by new evidence, and some of them are open to ethical objections. In general, I have not adopted them.

TABLE 3. *Endemicity in Australian bats*

Only groups that occur in Australia (and Tasmania) are considered. Aus. = Australia only. Aus. R = Australian Region (including New Guinea, adjacent islands, and East Indies east of Wallace's line). Aus.–O. = Australian Region into Oriental Region or beyond (including East Indies west of Wallace's line). Numbers in the table are of genera and of species groups as in Tate (1946), slightly modified.

Families	Genera			Species Groups		
	Aus.	Aus. R.	Aus.–O.	Aus.	Aus. R.	Aus.–O.
Pteropidae	0	3	2	0	7	3
Emballonuridae	0	0	2	1	1	1
Megadermatidae	1	0	0	1	0	0
Rhinolophidae	0	0	1	1	2	3
Hipposideridae	1	0	1	1	1	3
Vespertilionidae	0	1	8	1	3	8
Molossidae	0	0	1	1	0	1
Totals	2(10%)	4(19%)	15(71%)	6(15%)	14(36%)	19(49%)
Aus. R. total		6(29%)			20(51%)	
Aus. total		21			39	

endemicity for genera and for Tate's species groups in each family are given in table 3.

The seven chiropteran families of Australia proper are all widespread in Eurasia and, for some of them, other continents, and all are old. No fossils have been described from the Australian region but all of these families date as far back as middle Eocene to middle Oligocene in Europe. The fossils are few, and in fact all these families undoubtedly existed in the Eocene, and some may have originated in the Paleocene. They could, then, have reached Australia at any time since the early Tertiary, and there has been no differentiation of families and comparatively little of genera in the Australian Region.

The lower endemicity in comparison with the Muridae must be related to the greater ability of bats, flying animals, to cross water barriers. There are no strongly marked ecological barriers, aside from the water gaps, on the route from southeast Asia through the East Indies to New Guinea. All the islands are in the same climatic zone and most of them have considerable resemblance or, at least, overlap in other ecological conditions. The importance of that factor for Chiroptera is evident from comparison with the Neotropical Region. Although there is now no water barrier between the Neotropical and Nearctic Regions, the ecological barrier for bats is so marked that 56% of Neotropical families are endemic, as against none in Australia, and 87% of the Neotropical genera are endemic, as against only 29% in Australia. Bat distribution resembles that of birds and of many plants more than that of terrestrial mammals.

The Australian bats are much more diverse than the rodents, and, in sharp contrast to the latter, they represent an only moderately attenuated sampling of the whole of the original mainland Asiatic fauna. They do not fall into definite groups or waves as regards antiquity in the Australian Region but seem to have filtered in continuously throughout most of the Cenozoic. There is a wide spectrum of endemicity, which may be assumed to be approximately but only approximately correlated with antiquity in the region. Some Australian species, e.g., in *Myotis*, *Pipistrellus*, and *Miniopterus*, are very close to Indian, European, and even African species, although supposedly not quite identical. On the other hand there are a few clear-cut endemic Australian genera, notably the peculiar *Macroderma*, which is a predator on other bats. As is evident from table 3, the fruit bats or Megachiroptera (Pteropidae), although less diversified, are much more divergent in the Australian Region, much higher in endemicity, than the Microchiroptera (the six

other families). That suggests that the fruit bats are on an average older than the other bats in this region, although the first entry of microchiropterans may have been quite as early as this or earlier than that of the megachiropterans.

In spite of the absence of endemicity above the generic level, first colonizations could have been and probably were quite early in the Cenozoic. The antiquity of the families in Europe has been mentioned, and in spite of the paucity of fossil record even a living genus (*Rhinolophus*) now present in the Australian Region is reported from the Eocene of Europe. The total number of colonizations from the East Indies into the Australian Region has not been estimated, but it must have been large, on the order of twenty or thirty, possibly even more. The basic radiations, those now at family and in good part also generic level, occurred in Eurasia. There has been little true radiation in the Australian Region, but mostly local geographic differentiation below the generic level. The center of density in the region is not on the Australian continent but on the islands from New Guinea to the Solomons, where there are more groups of bats than in Australia proper. Only two of the twenty-one Australian genera and six of the thirty-nine species groups are confined to Australia. What regional differentiation there is clearly took place mostly in the islands (in part also the East Indian islands) before the groups reached continental Australia. Nevertheless two sharply distinct groups (*Macroderma* and *Rhinonicteris*) have originated in Australia, and most groups have become subspecifically to specifically distinct since reaching there.

For the bats, as for birds and such other animals as have spread along the whole chain, there is a gradient between the Asiatic and the Australian mainlands. Tate (1946) divided his species groups of bats according to assumed Asiatic or Australian regional origin, in a somewhat arbitrary way but using criteria of greatest distinction or differentiation, abundance, and presence of relatives toward one end or the other of the sequence. Some of his data on that basis are given in the upper part of table 4. He envisioned the gradients as representing two sequences of counter-migrations, one from Asia toward the Australian Region and one from the Australian Region toward Asia, both currents becoming more and more attenuated with greater distance from their assumed regions of origin. The same approach is implicit in earlier studies by Mayr (1944b)[4] and others, and it seems to be rather generally accepted.

That approach is certainly oversimplified, as has been realized by those who nevertheless followed it. It would not be a serious oversimplification for groups that did in fact have their basic differentiation and the origins of most of their taxa either in Asia or in Australia. That is true, for example, of marsupials, with virtually all taxa of Australian origin, as compared with placentals exclusive of bats and Muridae, with virtually all taxa of Asiatic origin. As suggested in fig. 1, those groups do show just the kind of overlap and opposite attenuations involved in the counter-migration hypothesis.

That hypothesis is, however, an unjustified oversimplification when applied to the bats. For them there are not two clear-cut and quite distinct centers of origin from which counter-migrations occurred. All the families, most of the genera (about 90%), and an undetermined but probably large number of the species groups of Australian bats originated outside that region, i.e., in the Oriental Region or beyond. The predominant pertinent movement was not in two counter-migrations, but in one direction, from Asia toward Australia. It thus seems probable that a significant element in the Australia–Asia gradient was increasing divergence or differentiation of animals really spreading in the opposite direction (Asia–Australia). During that movement they evolved progressively, in greatest part below the generic level, away from their Asiatic ancestry. The most impressive argument against that conclusion is that faunas

[4] Mayr's analyses did not include the bats, Tate's data being then not yet available.

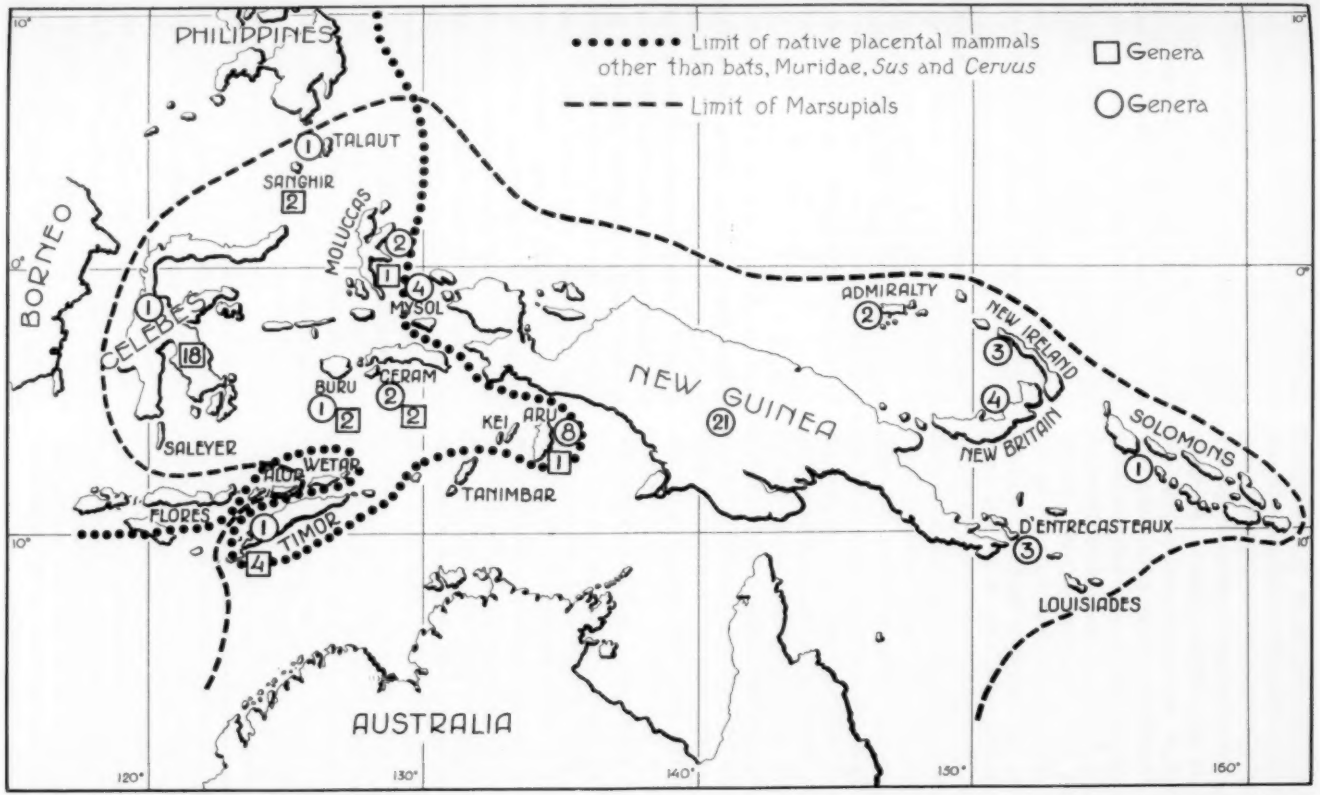


FIG. 1. Islands of the Australian Region, with numbers of genera and limits reached by marsupials and by native placentals other than bats, murids, and probable late human introductions of pigs and deer.

of intermediate islands are impoverished in comparison with either end of the sequence, and that ancestral forms or close relatives of many Australian species groups do not now occur in the Oriental Region. However, ancestors of those groups certainly did migrate through the islands even where relatives are now absent, and survival in the Oriental Region of recognizably close collaterals from the ancestry would not be expected in all or in many instances for migrations that started in the early Tertiary.[5]

On that hypothesis, the double sets of taxonomic clines of Tate and others are in fact a single set of genetic clines, with similarities decreasing and differences increasing in the one direction: Asia–Australia. By itself, that hypothesis is certainly also oversimple. There doubtless was also some counter-migration, involving low taxa, in the direction Australia–Asia. It must also be remembered that the principal eastern center of origin and dispersal was not Australia itself but New Guinea. There must moreover have been further low-level complications by origins and dispersals in various directions around other points scattered along the sequence.

The distributional facts can be more objectively shown by measures of actually existing faunal resemblances, which do not involve any hypotheses as to the places of origin of the various taxa. That approach is exemplified in the lower part of table 4. The figures show that if one starts at either more or less terminal area (continental East Indian islands; Australia and its continental islands), faunal resemblances become less with increasing distance. The measurement of that change has special interest for the particular case, but certainly the result is not surprising. The trend shown would almost inevitably appear (with different intensities) in comparisons of *any* two faunas with each other and with intermediate faunas. That result would arise in each of three models that are extreme or limiting cases and are not to be expected in pure, uncomplicated form in nature:

1. Counter-migrations between two areas originally with faunas completely different at the taxonomic levels under consideration.

2. Migration of the whole fauna from one terminal area to the other with progressive evolution along the way.

3. Local differentiation throughout a fauna that was originally uniform in and between the two areas.

In most cases of real faunas, all three factors probably act and interact, plus additional local perturbations and complications. That is doubtless true of the faunal relationships between the Oriental and Aus-

TABLE 4. *Taxonomic gradients of Chiroptera between the East Indies and Australia*

I. Percentages of species groups of supposed Asiatic and Australian (Region) origin, after Tate (1946).

"Origin"	Celebes	Moluccas	New Guinea and Solomons	Australia
Asia	90%	64%	35%	32%
Australian Region	10%	34%	65%	68%

II. Indices of faunal resemblance, $100C/N_1$ (see Simpson, 1960b), for species groups between the following areas:

A, East Indies, from Asia to edge of Asiatic continental shelf (modified Wallace's line; see Mayr, 1944b).

B, islands between A and C.

C, Australia and islands on its continental shelf (nearly but not quite to Weber's line; see Mayr, 1944b).

Raw data from Tate (1946).

	A-B	A-C	C-A	C-B
$100C/N_1$	58	31	31	53

[5] Comparatively small East Indian islands would be saturated with bats by a smaller number of stocks than those that reached the Australian Region and survived there. Later movements down the island chain could then occur only by the extinction (competitive or not) of earlier migrants on the smaller islands. Thus in spite of attenuation within each faunal wave and in spite of more westward islands having had larger totals of stocks through all time, at any one time (such as the present) each of the smaller islands would have a smaller number than had passed through it and survived in the Australian Region at the given time. It would also be precisely the earlier ancestral migrants, hence those whose descendants are now most distinctive in the Australian Region, that would have become extinct on the smaller intermediate East Indian islands.

tralian Regions. As regards the non-volant land mammals, the first factor predominates in the comparatively small area of overlap (Aru to Celebes). As regards the bats, such dominance of any one factor is less clear, but the second factor is probably at least as important as either of the other two.

For the bats, both Tate's counter-migration approach and the present approach by objective faunal resemblance place the 50-50 line, or faunal balance line, or Weber's line, east of the Moluccas rather than west of them, as has been calculated for several other groups of animals (Mayr, 1944b, and references there). It is only to be expected that different groups will differ somewhat in this respect, and all groups so far analyzed do agree in placing this significant line well to the east of Wallace's line.

Marsupialia

On the scale of this study, the distribution of the Australian marsupials is much simpler than that of the Muridae or Chiroptera. It has also been the subject of more reviews as well as detailed studies (e.g., and just by way of a few examples among many, Carter, Hill, and Tate, 1945; Laurie and Hill, 1954; Tate, 1952; Troughton, 1947). It will therefore be more briefly treated here.

In contrast with the Muridae and Chiroptera, there are some pertinent but still grossly inadequate fossil data for the marsupials. The Pleistocene to sub-Recent fauna of Australia, as on other continents, was richer than the present fauna, and it was already rather well-known in the 19th century (full lists and older literature in Simpson, 1930; later lists down to genera for both fossil and recent faunas in Simpson, 1945). Information on earlier mammals is still extremely scanty. Only about a dozen forms, scattered from late Eocene or Oligocene through the Pliocene, have as yet been described and not all of those are well identified.[6] (See Gill, 1957; Stirton, 1955, 1957a, 1957b.) All belong to Pleistocene or Recent families. As far as they go, they suggest that there has been some progression at generic and specific levels but that the general character of the Australian marsupial fauna did not change significantly from the mid-Tertiary, at latest, into the Pleistocene.

In conservative classification there are six living and two recently extinct families of Australian marsupials. A single species of *Phalanger* reaches Celebes and thus extends into an area not now usually included in the Australian Region (but still east of Wallace's line). With that trivial exception, all the marsupials of the Australian Region are endemic through the superfamily level. The major radiation clearly took place on the Australian continent and is surely very old. Scanty as it is, the fossil evidence strongly suggests that the families, and probably some or all subfamilies, were differentiated by the Miocene at latest, and probably much earlier. (The majority of Recent mammalian families in the rest of the world were differentiated in the Eocene and Oligocene; marsupial families may prove to be even older.)

From Australia marsupials have spread with a completely regular pattern of progressive attentuation (see fig. 1) as far as the Solomons to the northeast, Talaut to the north, and Celebes to the west. Four families and twenty-one genera occur in New Guinea, as listed in table 5. Most of the Papuan species and six of the genera are not present in Australia proper. Spread of their ancestors to New Guinea must have begun far back in the Tertiary, and there has evidently been some intermittent interchange ever since. Even though it has developed autochthonous genera, New Guinea has apparently not been a major center for marsupial evolution, as it was for both rats and bats. Nevertheless four genera shared with Australia perhaps evolved in New Guinea and may represent back-migration in Australia: the bandicoot *Echymipera*, the phalangers ("possums" to Australians) *Phalanger* and *Dactylopsila*, and the tree kangaroo *Dendrolagus*.

Ten of the Papuan genera have also

[6] The University of California group has other materials, partly studied by Stirton and Tedford but not yet published.

TABLE 5. *Marsupials of New Guinea*

Dasyuridae	
I A	*Phascogale* (including *Murexia*, *Antechinus*, *Neophascogale*, *Phascolosorex*)
I A	*Sminthopsis*
A	*Planigale*
I	*Myoictis*
A	*Dasyurus* (including *Satanellus*)
Peramelidae	
	Peroryctes, *Microperoryctes* } near Australian *Perameles*
I A*	*Echymipera*
A	*Thylacis* (= *Isoodon*)
Phalangeridae	
X A*	*Phalanger* (including *Spilocuscus*)
A	*Eudromicia*—near Australian *Cercaertus*
I A*	*Dactylopsila*
	Dactylonax—near Australian and Papuan *Dactylopsila*
I A	*Petaurus*
A	*Acrobates*
	Distoechurus
A	*Pseudocheirus*
Macropodidae	
I A	*Thylogale*
I A	*Protemnodon* (= *Wallabia*)
A*	*Dendrolagus*
I	*Dorcopsis* (including *Dorcopsulis*)

I —Extends also to nearby islands.
A —Also on Australia and probably originated there.
A*—Also on Australia but possibly originated on New Guinea.
X —Coextensive with Marsupialia on islands.

spread to other islands. They thin out rapidly and regularly with increasing distance from New Guinea. The most successful are *Phalanger*, which occurs wherever there are any marsupials in the Australian Region (and, as noted, as far as Celebes), gliding phalangers of the genus *Petaurus*, and bandicoots of the genus *Echymipera*. Significantly, all are typically rain forest groups. Ceram has an endemic genus of bandicoots, *Rhynchomeles*, which is most closely related to the endemic Papuan *Peroryctes*. It is clear that spread of all these groups to other islands has been from New Guinea and not directly from Australia. Even the Timor phalanger, *Phalanger orientalis*, although closer to Australia than to New Guinea, came from New Guinea, probably by way of Ceram, Buru, and Wetar. That species has spread from New Guinea in late Pleistocene or Recent times all over the islands that have marsupials, from the Solomons to Celebes, and also to Cape York Peninsula on Australia.

It is extremely unlikely that Australia had any nonvolant land placentals before the arrival of murids, which, as has been indicated, was no earlier than Oligocene and probably in the Miocene. By that time the basic marsupial radiation had already occurred, and its very extent supports the conclusion that the early marsupials had no placental competitors. Marsupials, abundant in the late Cretaceous and early Tertiary of North America and the early Tertiary, at least, of Europe, there faced strong and varied placental competition and became extinct by the Miocene (although one species later reinvaded the Nearctic). In South America specialized placental insectivores, rodents, and specialized placental carnivores, all absent in the earliest Paleocene of North America, were not among the original stocks derived from North America, and in their absence the marsupials underwent a partial radiation including much convergence toward those placentals. In Australia there were presumably no earliest Tertiary placentals and the marsupials underwent a radiation that was essentially complete ecologically.

The classical explanation for the absence of early placentals in Australia was that the marsupials evolved first and spread to that continent over a land bridge that was submerged before there were any placentals to cross it. Although that theory is still sometimes presented, it has long been abandoned by closer students of the problem. Marsupials and placentals, distinctly differentiated as such, apparently evolved at about the same time, and the earliest marsupials do not seem to have been significantly more primitive than the earliest placentals. The most reasonable explanation, although it is still speculative in the absence of any direct evidence, is that no land bridge was involved but that primitive marsupials reached the continent over a series of sea barriers. The

original stock must have been of small, arboreal, opossumlike (i.e., didelphoid, not phalangeroid) animals such as would be particularly apt at island-hopping. Arrival of one pair or of a single gravid female in Australia could have sufficed. Subsequent extinction of marsupials in the East Indies (where fossil faunas that might include early marsupials are completely unknown), would be no more surprising than their known extinction in Eurasia.[7]

Under this theory, the placentals were excluded by the sequence of marine barriers that the marsupials crossed. That may have been because southeastern Asiatic placentals of that early time were less apt at island-hopping. Somewhat more likely is the hypothesis that the event was extremely improbable for either group and that selection of one group rather than another for the unique, improbable event was purely by chance (or was stochastic). As has been shown, similar events repeatedly occurred for one group of placentals, the murids, from about mid-Tertiary onward. Of south Asiatic mammals the murids (not yet in existence when the marsupials reached Australia) are clearly particularly well adapted for island-hopping. It is also possible that the barriers became somewhat less formidable around Miocene times.

The time of arrival of marsupials in Australia was probably late Cretaceous or Paleocene. There is suggestive although not wholly adequate evidence from North America and Europe that the marsupials were not clearly differentiated as such until the late Cretaceous. From the other direction, the great scope of marsupial radiation in Australia suggests very early arrival there. The earliest fossil marsupial known from Australia, *Wynyardia bassiana*, is of late Eocene or Oligocene age and already belonged to a highly specialized Recent subfamily and family (Gill, 1957).

[7] No fossil marsupials have been found in Asia, but there too faunas that might include them are as yet very poorly known. Known presence of early marsupials in both Europe and North America makes it almost certain that they also occurred in Asia.

A possible but on the whole considerably less probable theory is that pre-marsupial therians (perhaps some branch of the Pantotheria) reached Australia in the earlier Cretaceous, or even in the Jurassic, and there evolved in isolation into marsupials. In that case, also, the group island-hopped in the late Cretaceous, but in the reverse direction from Australia to Asia whence it spread to Europe and to the Americas.

Monotremata

As is well known, the monotremes belong to two families, the Tachyglossidae with *Tachyglossus* on both Australia and New Guinea and its close ally *Zaglossus* on New Guinea only, and the Ornithorhynchidae with the single Australian genus. These are extreme endemics, representing a whole subclass now confined to the Australian Region. Universal recognition that they have some very primitive characters in comparison with other (or true) mammals has sometimes obscured the fact that in other respects they are extremely specialized, about as specialized as any mammals (or reptiles). The two families are also markedly divergent from each other and must have been separated for a very long time.

A few Australian Pleistocene fossils cast no light on the history of the group. In the rest of the world nothing possibly related to them is known in the late Mesozoic or the Cenozoic. The one possibly direct clue, a slender and equivocal one, is that the morganucodonts or eozostrodonts, known from the late Triassic of Great Britain, share a peculiarity of the ear region with the recent monotremes. On that account Kermack and Mussett (1958) have suggested that the morganucodonts are, or are near, the monotreme ancestry. That is quite possible, but the enormous gap both in time and in known morphology makes it extremely tentative at present. The morganucodonts were probably a lineage from therapsids, nominal reptiles, that was acquiring mammalian structural status parallel to but quite separately from the contemporary ancestry of the Theria (marsupials and placentals, to-

gether). It is also possible that they were an offshoot from the therian ancestry near the nominal reptile-mammal boundary. Regardless of the involvement of the morganucodonts, other less possibly direct but more abundant and compelling comparative anatomical evidence suggests that the monotremes were derived from the therapsids either quite separately from the therian ancestry or at most from near the very base of that ancestry at a stage equivocal in status between reptiles and mammals. Whether they should, indeed, continue to be classified as mammals, which I consider still most convenient, becomes then a mere matter of definition (see, e.g., Simpson, 1960a).

The most probable present hypothesis on the geographic history of the monotremes is that advanced therapsids or early post-therapsids, near the arbitrary reptile-mammal line as usually drawn, reached Australia in the late Triassic or in the Jurassic, that they there gave rise to the monotremes of stricter definition, and that the latter have persisted in and have always been confined to the Australian Region. It is completely speculative, but is an interesting speculation, that there may have been a Mesozoic monotreme radiation in Australia that was mostly, yet not entirely, replaced by a later, mainly early Tertiary, marsupial radiation.

Further Problems

It is certainly not claimed that this study of the broadest aspects of the historical zoogeography of Australian mammals, departing from the basis of work by Tate and many others, is definitive. It does, however, seem improbable that this more general aspect of the subject can be carried much further on the basis of recent animals. What is needed to fill in detail and to check present hypotheses and speculations is fossil evidence. The dozen or so Tertiary mammals so far described, interesting as they are in detail, throw virtually no light on the broad problems. Especially needed are Miocene and Pliocene microfaunas including murids, any Tertiary faunas including bats, and Cretaceous-Paleocene faunas of marsupials. Intense efforts so far (e.g., by Stirton and his associates) have had quite limited success, but enough to encourage the belief that such faunas can eventually be found. Discoveries of early Tertiary faunas in southern Asia and the East Indies may also throw useful light on the Australian Region.

On mammalian distributions within Australia, not treated here, some suggestive work has been done, but less than on some other groups (e.g., certain birds, frogs), and more remains to be done. Here there are available rather extensive Pleistocene and sub-Recent data, but there is great need for the discovery and collection of more small mammals, especially, of those ages. It may be salutary to point out that in North America the accumulation of Pleistocene-Recent data of that desirable sort is now enormous, and yet that the progress so far made on its evolutionary, historical, zoogeographical, and ecological interpretation is highly unsatisfactory.

Summary

1. The five orders of native Australian mammals have had characteristically different geographic histories.

2. The dingo, the only native placental carnivore, is probably a feral species of prehistoric human introduction.

3. The Muridae have made multiple invasions of the Australian Region from Asia across the stepping stones of the East Indian islands, beginning probably in the Miocene. An "old Papuan" group now includes five rather isolated genera in New Guinea. Another old Papuan stock evolved there into the peculiar subfamily Hydromyinae. There have been two spreads of hydromyines, one early and one late, from New Guinea into Australia. The supposed hydromyines of the Philippines are only convergent toward the true Hydromyinae.

4. A stock probably of old Papuan origin but not positively identified in New Guinea early reached Australia and radiated extensively there into the *Pseudomys* group of genera, which warrants recognition as a sub-

family Pseudomyinae. There was one late invasion of this group into New Guinea.

5. A probably somewhat later (Pliocene?) migrant into New Guinea gave rise there to the *Uromys* group of four genera. Thence first *Melomys* and later *Uromys* reached Australia, with little further differentiation.

6. There was a later, late Pliocene or early Pleistocene, invasion of New Guinea by one or two partly differentiated lineages of *Rattus*. This stock speciated considerably in New Guinea, reached Australia, and speciated more widely there. Prehistoric and historic introductions by humans followed.

7. The bats of the Australian Region are more varied than the rodents but have less endemicity. All were also derived ultimately from Asia, where the basic differentiation occurred, in a long series of migrations, perhaps thirty or even more, from early Tertiary onward.

8. New Guinea was the major center for bat differentiation within the Australian Region, mainly at lower taxonomic levels. Repeated spread from New Guinea to Australia occurred. There was no extensive radiation in Australia, and new differentiation there was less than in New Guinea.

9. There is a fairly regular gradient of chiropteran faunal similarity from Asia through the East Indies to Australia. Migration was predominantly in the direction Asia–Australia, and the gradient is in part due to progressive evolution along that line, in part to subsequent counter-migration from New Guinea westward, and in part to more local differentiations and movements.

10. Marsupials probably reached Australia in the late Mesozoic or earliest Cenozoic by island-hopping through the East Indies, where they became extinct. The great, well-known marsupial radiation occurred in Australia. Numerous marsupials spread from Australia to New Guinea, where there was some further progressive differentiation but little true radiation. There was some back-migration from New Guinea to Australia. Continuously attenuated spread to other islands, as far as the Solomons, Talaut, and Celebes, occurred from New Guinea, not directly from Australia.

11. Ancestors of the monotremes, still near the nominal reptile-mammal line, probably reached Australia in the Jurassic or late Triassic and there evolved into the Monotremata, strictly speaking, which have probably never occurred outside the Australian Region.

Literature Cited

Carter, T. T., J. E. Hill, and G. H. H. Tate. 1945. Mammals of the Pacific world. Macmillan, New York.

Darlington, P. J. 1957. Zoogeography. The geographic distribution of animals. Wiley, New York.

Gill, E. D. 1957. The stratigraphical occurrence and paleoecology of some Australian Tertiary marsupials. Mem. Nat. Mus. Victoria, no. **21**: 135–203.

Kermack, K. A., and F. Mussett. 1958. The jaw articulation of the Docodonta and the classification of Mesozoic mammals. Proc. Roy. Soc. London, B, **148**: 204–215.

Laurie, E. M. O., and J. E. Hill. 1954. List of land mammals of New Guinea, Celebes, and adjacent islands. British Museum, London.

Mayr, E. 1944a. Timor and the colonization of Australia by birds. Emu, **44**: 113–130.

——. 1944b. Wallace's line in the light of recent zoogeographic studies. Quart. Rev. Biol., **19**: 1–14.

Raven, H. C. 1935. Wallace's line and the distribution of Indo-Australian mammals. Bull. Amer. Mus. Nat. Hist., **68**: 177–293.

Simpson, G. G. 1930. Fossilium catalogus. 1: Animalia. Part 47. Post-Mesozoic Marsupialia. W. Junk, Berlin.

——. 1945. The principles of classification and a classification of mammals. Bull. Amer. Mus. Nat. Hist., **85**: i–xvi, 1–350.

——. 1950. History of the fauna of Latin America. Amer. Scientist, **38**: 361–389.

——. 1960a. Diagnosis of the classes Mammalia and Reptilia. Evolution, **14**: 388–392.

——. 1960b. Notes on the measurement of faunal resemblance. Amer. Jour. Sci., **258A**: 300–311.

Stirton, R. A. 1955. Late Tertiary marsupials from South Australia. Records South Australian Mus., **11**: 247–268.

——. 1957a. A new koala from the Palankarinna fauna of South Australia. Records South Australian Mus., **13**: 71–81.

——. 1957b. Tertiary marsupials from Victoria, Australia. Mem. Nat. Mus. Victoria, no. **21**: 121–134.

TATE, G. H. H. 1936. Some Muridae of the Indo-Australian region. Bull. Amer. Mus. Nat. Hist., **72**: 501–728.

——. 1946. Geographical distribution of the bats in the Australian archipelago. Amer. Mus. Novitates, no. 1323.

——. 1951. The rodents of Australia and New Guinea. Bull. Amer. Mus. Nat. Hist., **97**: 183–430.

——. 1952. Mammals of the Cape York Peninsula, with notes on the occurrence of rain forest in Queensland. Bull. Amer. Mus. Nat. Hist., **98**: 563–616.

TROUGHTON, E. 1947. Furred animals of Australia. Scribner's Sons, New York.

FREQUENCIES AND ALLELISM OF LETHAL FACTORS WITHIN AND BETWEEN GENE ARRANGEMENTS. I. SAN JACINTO MOUNTAINS[1]

CARL EPLING, VICTOR E. TINDERHOLT,[2] AND R. H. T. MATTONI
Department of Botany, University of California, Los Angeles

Received January 30, 1961

This report will subsume the differences in frequency of lethal chromosomes among certain inversion rearrangements of the third chromosome of *Drosophila pseudoobscura*, together with their degrees of allelism in different environments. This first paper will deal chiefly with arrangements found in the San Jacinto Mountains, southern California. A second will deal chiefly with arrangements found in Guatemala. A third will compare mutation rates between certain arrangements.

Populations of *Drosophila pseudoobscura* are known to carry a considerable load of recessive autosomal lethals (Wright, Dobzhansky, and Hovanitz, 1942). Furthermore, the third chromosome of this species has undergone a series of inversions which have resulted in 22 different gene arrangements. Crossing over between the heterozygotes of these arrangements is virtually suppressed (Dobzhansky and Epling, 1948; Levine and Levine, 1954). A change of arrangements may also significantly change the frequencies of crossing over at certain loci in different structural homozygotes (unpublished data). Thus, each arrangement tends to be preserved in its population as a discrete and different linkage group. Finally, the several arrangements occur at different frequencies in different environments within the species range (Dobzhansky and Epling, 1944). Each arrangement fluctuates in frequency at a given collecting site, but the pattern of their relative frequencies has remained not dissimilar for many generations at the sites that have been systematically sampled (Epling and Lower, 1957). Thus, the Standard arrangement has remained the most frequent and the Treeline arrangement has been the least frequent of the five principal arrangements in the San Jacinto Mountains, southern California, since 1939. This period would embrace more than 100 and perhaps as many as 200 breeding cycles or "generations" if we allow six generations per year, which is probably a minimal number. In Guatemala, however, where the Standard arrangement has not been found, Treeline has a frequency regularly four or five times greater than in southern California. At the same time, the Santa Cruz arrangement is the dominant arrangement in Guatemala, taking the place of Standard in California, whereas in California its frequency is negligible. The structural relations of these arrangements have been described and illustrated by Dobzhansky (Dobzhansky and Epling, 1944).

Heretofore, the frequencies of lethals and their alleles have been determined without reference to the gene arrangements with which they are associated. But the frequency patterns of the arrangements are apparently related to differences of environment not only over wide areas (Epling in Dobzhansky and Epling, 1944), but even within relatively short geographic distances, such as those along a transect in the San Jacinto–Santa Rosa Mountains (Epling, Mitchell, and Mattoni, 1957). The assumption is reasonable then that these frequency patterns are adaptive for each population and are controlled by natural selection in each environment. The frequency of each arrangement might therefore depend on the genetic factors that it contributes to the modal genome of a population in each environment. Among the factors involved in this

[1] A series supported by Contract C-3032, National Institutes of Health, dedicated to Dr. Leslie C. Dunn, long time Evocative Professor of Zoology at Columbia University, on the occasion of his retirement.

[2] Died in his laboratory, aged 32, Nov. 20, 1961.

relation may be the frequencies of lethals and their alleles. It has seemed desirable, therefore, to ascertain whether differences of frequency and allelism of lethal chromosomes exist among the arrangements of a population, and whether those of one population are similar to those of another. To do this we have been comparing the lethal arrangements in the San Jacinto Mountains with those in Guatemala over a period of several years.

The San Jacinto population referred to here is a composite one obtained from two collecting sites, at Pinon Flat and at Vandeventer Flat. The pattern of arrangement frequencies has been consistently somewhat different at each site. Both sites have been described and illustrated elsewhere, most recently in Epling, Mitchell, and Mattoni (1957). They are about five miles apart, but represent two sites along an ecological cline from the desert to forested mountains. So far as lethal frequencies and allelism are concerned these populations are statistically homogeneous. The Guatemalan site, 25 km from Guatemala City on the Antigua Road, and its notably different ecology (Epling in Dobzhansky and Epling, 1944) will be described in a subsequent publication.

Methods

This study was begun in 1953, a favorable year for collections from February to October at Vandeventer Flat and from February to May at Pinon Flat. The area sampled at Pinon Flat was substantially the same as that sampled earlier by Dobzhansky by means of a similar trapline of baited cups. The area sampled at Vandeventer Flat was comparable in size. During 1955 and subsequently we have used baited 2 gallon cans arranged loosely in a much smaller area at each site. In the first instance collections were made at intervals during the brief evening period of collecting of one day. In the second, the cans have been left for several days before collecting. Some flies seem to remain in the cans overnight or perhaps for several days, which they do not do in the cups. Thus, the population sampled may differ by each method. If so, the arrangement frequencies seem not to have been altered, and the collections have been larger.

The arrangements names to be used will be abbreviated as follows: Standard (ST), Arrowhead (AR), Chiricahua (CH), Treeline (TR), Pikes Peak (PP), Santa Cruz (SC), Oaxaca (OA), and Cuernavaca (CU).

Our experiment has been so designed that flies collected in the evening of one day are processed in the laboratory on the following morning. During most of the experiment only males were used. Each male was mated to several virgins of a marker stock which carried *orange* and *purple* mutants on a ST arrangement $\frac{or\ pr\ (ST)}{or\ pr\ (ST)}$. Salivary glands of eight larvae from each mating were used to determine the frequencies of the arrangements. The male offspring of these matings were used to extract the lethal chromosomes present. More recently, when samples have been small because of drought, a single larva from each female was also used to determine frequencies, and her sons were then used to extract lethals.

The identification of the arrangements having been made, an adult male from each vial was then crossed to several virgins of an *orange-Lobed* stock carried on the SC arrangement, balanced over a wild type lethal CU, $\frac{or\ Lb\ (SC)}{1(CU)}$. Lobed is lethal when homozygous. Three or four pairs of $\frac{or\ Lb}{+}$ offspring were selected from each cross, were put into vials and allowed to breed at 22° C. The single wild arrangement that each strain would now carry was then determined from the emerging larvae. The proportion of *Lobed* to wild type in each vial producing mature flies was then ascertained. If the proportion of wild type to *Lobed* was 15% or less, the strain was retested until sufficient numbers had been counted to make a clear decision as to lethality. If, finally, in a total of 100 or more F_3 or F_4 retest progeny, 0–2% were wild type the original chromosome was classed as lethal; if 2–15% as semilethal, and if more than

15%, as nonlethal. To maintain the stocks used later in the allelism tests, each of which carried a wild chromosome, they were balanced over a marker chromosome according to their kind in respect of possible crossing over. The arrangements of the Standard phylad, ST, AR, and PP, were crossed to $\frac{or\ Lb\ (SC)}{1\ CU}$. *Lb* is a homozygous lethal. Those of the SC phylad were crossed to $\frac{or\ Bl\ Scu\ pr\ (ST)}{1\ CU}$. *Bl* is a homozygous lethal. Later, *Lb* and *Bl* were combined on both ST and SC, which markers were then used in the same way.

The allelism tests made on the 1953 stocks from Pinon Flat and Vandeventer Flat were carried out in 1953 and 1954. They were conducted in vials in the usual way by systematic intercrosses, using the same criteria for lethality. All suspected cases of allelism were retested by adequate numbers of progeny. Some stocks were lost before retests were completed. Certain of the TL lethals of 1953 were also crossed to those obtained in 1955 and 1956, as will appear below.

Results

The numbers and frequencies of the arrangements in the samples of 1953 from which the lethal chromosomes were extracted are as follows:

	Pinon Flat		Vandeventer Flat	
	N	f	N	f
ST	1842	0.502	1089	0.533
AR	756	0.206	416	0.205
CH	492	0.134	195	0.096
TL	230	0.063	140	0.069
PP	336	0.092	181	0.089
	3656	0.997	2021	0.997

The deficiency of .003 results in each case from arrangements of low frequency, such as Santa Cruz, which are not listed.

The numbers and frequencies of chromosomes tested for lethality in the combined samples from Pinon and Vandeventer Flats are shown in table 1. When these tests for lethality were being made in 1953 every effort was made to secure evidence of lethality for both arrangements present in each male. Both arrangements could not be tested in the homozygotes and some tests were lost. Nevertheless about 70% of the arrangements in the whole sample were tested. The data presented here are accordingly fairly representative of the situation in this population. The samples were collected regularly throughout the season when collecting was profitable. No significant differences in the frequencies of lethal chromosomes were found from month to month or seasonally, nor between these sites. For our present purpose we are accordingly regarding them as comprising one population.

The left half of table 1 shows that the array of lethal frequencies is not statistically homogeneous and that the chief contributors to nonhomogeneity are TL and PP, the former being significantly high and the latter significantly low. At the same time there appears to be no consistent relation between the frequencies of lethal arrangements and the frequencies of the arrangements themselves. The array of semilethal frequencies, on the other hand, is homogeneous. A comparison of these arrays suggests that the conditions for lethality and for semilethality are not necessarily the same.

Table 2 has been introduced at this point to provide a comparison with similar data from Guatemala, where TL is one of the more frequent arrangements with a frequency several times that in California. The data from California pertain to the monthly samples of only one year; those from Guatemala pertain to two successive years, 1958 and 1959, and the data for 1960 are now being analyzed. Inspection of this table will show, first, that the array of frequencies of lethal arrangements, as defined above, was statistically homogeneous in 1958 and nonhomogeneous in 1959. TL was the principal contributor to nonhomogeneity in 1959, and the frequencies of its lethal arrangements were also high in 1958. It shared its position of high frequency in 1958 with CU, an arrangement of consistently low frequency in this population, just as TL in

TABLE 1. *Number and frequencies of lethal and semilethal chromosomes in the gene arrangements tested (Pinon and Vandeventer Flats, 1953)*

Arrangements			Lethal frequencies					Semilethal frequencies				
Frequency		Number tested	Lethals	Probable[1] lethals	Totals	f and 2 σ range	P[2]	Semi-lethal	Semi-lethal visibles	Totals	f and 2 σ range	P[3]
ST	.516	1064	138	6	144	.135(.114–.156)	.3	22	1	23	.0216(.017–.026)	↑
AR	.206	528	84	1	85	.161(.129–.193)	.7	17	1	18	.0341(.025–.044)	
CH	.121	266	37	2	39	.147(.103–.190)	.5	7	0	7	.0263(.015–.041)	.5
TL	.065	181	53	1	54	.298(.230–.366)	.0001	3	0	3	.0166(.001–.033)	
PP	.091	246	6	3	9	.041(.022–.057)	.0001	4	0	4	.0163(.002–.030)	↓
Totals	.999	2285	318	13	331	.145		53	2	55	.024 (.023–.025)	

[1] Strains lost before retesting was complete but after not less than 40 *Lb* and no wild type flies had been counted.
[2] Homogeneity calculated for the array from chi-square values.
[3] Homogeneity calculated for the array from the Poisson distribution.

California. These data accordingly suggest, again, that the frequencies of lethal arrangements bear no direct relation to the frequency in a population of the arrangements themselves; and that TL may generally carry more lethal chromosomes than others. The array of semilethal chromosomes, unlike that in California, is nonhomogeneous (for both years), and TL is the principal contributor to nonhomogeneity. Thus, the data in this table suggest that the conditions for lethality and semilethality may also differ in two widely separated populations which are controlled by opposed climatic factors (Epling in Dobzhansky and Epling, 1944, fig. 2).

We turn now to allelism within and between lethal chromosomes collected at Pinon Flat and Vandeventer Flat in the San Jacinto Mountains, confining ourselves first to ST and AR and then to TL. The degree of allelism within and between ST and AR at both sites were tested by the series of crosses shown in table 3. No significant dif-

TABLE 2. *Numbers and frequencies of lethal and semilethal chromosomes in the gene arrangements tested (Guatemala, 1958 and 1959)*

Arrangements			Lethal frequencies					Semilethal frequencies				
	f	Number tested	Lethals	Probable[1] lethals	Totals	f and 2 σ range	P[2]	Semi-lethal	Semi-lethal visibles	Totals	f and 2 σ range	P[3]
1958:												
SC	.422	192	53	0	53	.276(.211–.341)	↑	6	0	6	.031 (.010–.052)	↑
TL	.264	120	36	2	38	.317(.232–.402)		8	2	10	.083 (.046–.117)	
OA	.262	119	22	2	24	.202(.128–.276)	.3	4	1	5	.042 (.017–.076)	.05
CU	.053	24	8	0	8	.333(.141–.525)	↓	0	0	0[4]	.016 (.000–.120)	↓
T	1.001	455	119	4	123	.270		18	3	21	.046	
1959:												
SC	.455	168	33	0	33	.196(.135–.257)	↑	4	1	5	.030 (.099–.048)	↑
TL	.205	88	29	0	29	.329(.226–.426)	.01–	7	2	9	.102 (.057–.148)	
OA	.294	118	17	0	17	.144(.079–.209)	.02	3	1	4	.034 (.008–.059)	.02
CU	.045	37	7	0	7	.189(.060–.318)	↓	0	0	0[4]	.016 (.000–.052)	↓
T	.999	411	86	0	86	.185		14	4	18	.044	

[1] Lost before testing was complete but after not less than 40 *Lb* and no wild type flies had been counted.
[2] Homogeneity for the array calculated from chi-square values.
[3] Homogeneity for the array calculated from the Poisson distribution.
[4] Omitted from the test for homogeneity.

TABLE 3. *Allelism of lethal chromosomes of ST and AR in 1953 at Pinon and Vandeventer Flats (above) and between arrangements and sites (below)*

Arrangements crossed	N chromosomes tested	Possible intercrosses [K(K−1)/2]	Intercrosses made	Lethal chromosomes found		Alleles found
				N	f	
PST × PST	362	1653	1323	58	.160	4
VST × VST	649[1]	2701	2181	74	.148	11
PAR × PAR	189	378	378	28	.148	5
VAR × VAR	309[2]	1275	858	51	.165	2
	1509	6007	4740	211	.140	22
PST × VST		4292	2765			11
PAR × VAR		1428	1106			7
PST × PAR		1624	1230			9
VST × VAR		3774	2171			11
PST × VAR		2958	1748			7
VST × PAR		2072	1332			6
		16148	10352			51

[1] 53 tests lost.
[2] 30 tests lost.

ferences were found in the frequencies of the lethal chromosomes in the two arrangements at the two sites, nor in the degree of allelism. The whole array can be regarded as statistically homogeneous, so far as allelism is concerned. Similar tests were made on 181 TL chromosomes from both sites in which 54 lethal chromosomes were found and 53 were cross-tested (line 1 of table 5). The degree of allelism in ST and AR, combined, is compared with that of TL in table 4 and in fig. 1.

Table 4 is organized to indicate the numbers of lethal chromosomes in each of several allelic groups. Thus, 130 of the 211 of the cross-tested lethal chromosomes of ST and AR (table 3, column 5) were found to have no alleles; 17 were found to have 1 allele, 4 were found to have 2 alleles, and so on. None of the chromosomes in one group was allelic to those in another. In the case of TL, 12 had no alleles, 2 had 1 allele, 1 had 5 alleles, and so on up to 11. The degree of allelism is clearly different in the two categories. These differences are graphed in fig. 1. The 17 instances with one allele in ST and AR are represented by diagram (a), the 4 instances with 2 alleles by the upper drawing in diagram (b), the 6 instances with 3 alleles by diagram (c), and so on. The two instances in TL with 2 alleles are represented in diagram (m), and those with 5, 8, 9, and 11 alleles are represented in diagrams (m) and (O).

The notation of fig. 1 is as follows. Each numbered letter represents a lethal chromosome among the 211 or 53, as the case may be. S and s indicate ST arrangements; A and a indicate AR arrangements; T and t indicate TL arrangements. The capitals represent those from Pinon Flat, the lower

TABLE 4. *Numbers of chromosomes with 0 or more alleles in common within and between ST and AR and within TL (1953). Weighted values are shown in parentheses (see text)*

Alleles in common by group	Numbers of allelic groups (see text)								
	0	1	2	3	4	5	8	9	11
ST + AR (211)	130	17(22)	4(8)	6(5)	1(0)	1(0)			
TL (53)	12	2				1	1	1	1

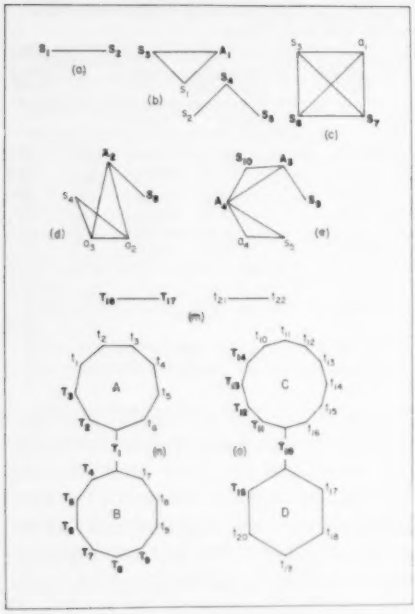


FIG. 1. The patterns of allelism between lethal chromosomes within and between the Standard and Arrowhead arrangements, and within Treeline, San Jacinto Mountains, 1953 (see text).

case represents those from Vandeventer Flat. Chromosomes with allelic factors are joined by the lines. Thus, chromosomes S_1 and S_2 carry a lethal factor each of which is allelic to the other, but to no others in the array. Similarly with S_3, A_1 and s_1; and with s_3, a_1, and S_6 and S_7. In the lower drawing of diagram (b) and in diagrams (d) and (e) allelism is partial; that is S_4 is allelic to s_2 and to S_5, but the two latter were found to be nonallelic after adequate retesting. Similarly with the groups diagrammed at (d) and at (e). Thus, A_2 is allelic to a_2 and a_3, and is also allelic to S_8, but S_8 is nonallelic to a_2 or a_3 and s_4. These data recall a situation in *D. melanogaster* from different populations described by Hinton (1957). However, all of the factors in diagrams (m), (n), and (o) are completely interallelic within each group. The interconnecting lines that would be comparable to those in diagram (c) were omitted for clarity.

The simplest explanation of the partial allelism in diagrams (b), (d), and (e), is that some lethal chromosomes carry two factors, presumably at different loci, of which one is allelic to a factor on one chromosome, the other being allelic to a factor on another chromosome. In this case, S_4, A_2, a_2, a_3, A_3, and A_4, that is 6 chromosomes of the 211 tested, would each carry two factors. Similarly in TL, 2 chromosomes of 53 carry 2 factors (T_1 and T_{10}). This assumption is being tested. With this explanation in mind, however, one may readily see that the lower drawing in (b) actually represents two examples similar to the example shown in (a). Diagram (d) can be resolved into 1 similar to that of (a) and 2 similar to that in the upper drawing of diagram (b); likewise with diagram (d). If we regard all of the partial cases of allelism found as being compounded of two or more simpler cases (as above), the numbers of allelic groups in table 4 will be changed to the numbers in parentheses. There would be, then, 165 different lethal loci carried by the 211 ST and AR chromosomes, and 20 different lethal loci carried by the 53 TL chromosomes.

The greater degree of allelism in TL results chiefly (about 70%) from the four recurring factors indicated as A, B, C, and D in fig. 1, diagrams (n) and (o), which pertain only to the samples of 1953. Sampling since then has continued regularly each year. During this period the numbers of TL chromosomes obtained has steadily diminished, partly because of a sustained drought which has decreased the samples and partly because of a decrease in the frequency of TL. Nevertheless we have been able to compare the frequencies in this arrangement of factors A, B, C, and D in several temporal populations over a period of perhaps 25 generations (table 5). The data in this table indicate that these frequently recurring factors have persisted at substantially the same frequencies during this period, and that whatever balance that may exist between their mutation rates and selection has not changed significantly.

The assumption of two lethal factors on

TABLE 5. *Frequencies and allelism of TL lethals in successive years in combined samples from Pinon and Vandeventer Flats*

Year	Chromosomes tested	Lethal chromosomes		N tested for allelism	N of chromosomes allelic within four allelic groups				f of chromosomes with at least one common lethal
		N	f		A	B	C	D	
1953	181	54	.298	53	6[1]	12[1]	9[2]	10[2]	.66
1955	45	10	.222	9	4	1	1	0	.67
1956	80	19	.236	17	4	3	1	2	.59

[1, 2] One factor in common in each case (fig. 1).

some chromosomes but not on others can be verified only by appropriate tests that locate the relative positions of the loci involved, allelic and nonallelic. To determine whether the higher frequencies of lethals and semilethals in TL result from a higher mutation rate or from a low selection pressure, or for other reasons, can be determined only by appropriate tests. These tests are in progress and will be reported in a subsequent paper.

SUMMARY

1. A comparison is made of frequencies and allelism of lethal factors in the principal gene arrangements of the third chromosome of *Drosophila pseudoobscura* from the San Jacinto Mountains, southern California, together with a comparison of frequencies in a Guatemalan population where the Treeline arrangement also occurs but at a much higher frequency than in California.

2. The frequencies of lethal chromosomes in 1953 in the San Jacinto populations were different in the several arrangements. Those of the Treeline arrangements were significantly high; those of the Pikes Peak arrangement were significantly low. The frequencies of the semilethal chromosomes were not significantly different.

3. Treeline lethal chromosomes were relatively high in frequency in the Guatemalan population in both 1958 and 1959, and were the principal contributor in 1959 to nonhomogeneity of the array of arrangements tested. This was likewise true of the semilethals in both years.

4. The patterns of allelism differ between certain arrangements. The chromosomes allelic to each other fall into several groups according to the number of alleles in each group. In the Standard and Arrowhead arrangements the number of alleles within a group was not more than 4. In the Treeline arrangement in California it was as many as 12. Thus, about 70% of the Treeline lethal chromosomes in the Californian population are attributable to four commonly recurring lethal factors.

5. Some allelic groups in the Standard and Arrowhead arrangements are not completely and regularly allelic. The data suggest that this partial allelism may result from the fact that some chromosomes carry one factor allelic to a factor in some chromosomes, and another allelic to one in other chromosomes.

6. Comparison of the lethal and semilethal frequencies in the two populations suggests that the Treeline arrangement is generally characterized by a high frequency of lethals, regardless of its own relative frequency and the kinds of associated arrangements; and that the conditions for its lethality and semilethality differ from year to year and from site to site.

LITERATURE CITED

DOBZHANSKY, T. 1939. Genetics of Natural Populations. IV. Mexican and Guatemalan populations of *Drosophila pseudoobscura*. Genetics, **24**: 391–412.

—— AND C. EPLING. 1944. Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Washington, Publ. **554**.

—— AND ——. 1948. The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci., **34**: 137–141.

EPLING, C., D. F. MITCHELL, AND R. H. T. MATTONI. 1955. Frequencies of inversion combinations in the third chromosome of wild males of *Drosophila pseudoobscura*. Proc. Am. Acad. Sci., **41**: 915–921.

——, ——, AND ——. 1957. The relation of an inversion system to recombination in wild populations. EVOLUTION, **11**: 225–247.

—— AND W. R. LOWER. 1957. Changes in an inversion system during a hundred generations. EVOLUTION, **11**: 248–256.

HINTON, T. 1957. An analysis of naturally occurring lethals. Genetics, **42**: 376–377 (Abst.).

LEVINE, R. P., AND E. E. LEVINE. 1954. The genotypic control of crossing over in *Drosophila pseudoobscura*. Genetics, **39**: 677–691.

WRIGHT, S., T. DOBZHANSKY, AND W. HOVANITZ. 1942. Genetics of natural populations. VII. The allelism of lethals in the third chromosome of *Drosophila pseudoobscura*. Genetics, **27**: 363–394.

FLORAL EVOLUTION IN THE FAMILY GRAMINEAE

H. T. Clifford
University of Queensland, Brisbane, Australia

Received February 20, 1961

With few exceptions the flowers of plants belonging to the family Gramineae are small and have only a rudimentary perianth. In what follows the variation in floral structure within the family is described and it is shown that most of the known floral types may be derived by reduction from an *Arundinaria*-type flower. A method for calculating the total possible range of variability is presented, and the numbers of known and theoretically possible flowers are compared for several different types of flowers. The consequences of floral evolution on the present-day reproductive mechanisms within the family is briefly discussed.

Floral Types in the Gramineae

As with the flowers of most monocotyledons, those of the Gramineae are usually subtended by a bract (lemma) and bracteole (palea). The perianth when present consists of a single trimerous whorl above which occur the essential organs. As the perianth members alternate with the stamens of the lower whorl when these are present, they are regarded as petals rather than sepals. It was thought by earlier botanists (Lindley, 1846) that the palea and lemma might represent a sepaline whorl, but few would now accept this interpretation, though it has recently been advocated again in a modified form by Stebbins (1951).

Except in a few genera such as *Streptochaeta*, where it may attain a length of a centimeter or more, the perianth is very short and is included within the palea and lemma. The individual perianth members are called lodicules and they may be either membranous or fleshy. When present the lodicules are either 2 or 3 in number and are generally free from each other. In the few species which appear to have a single large anterior lodicule it has been shown, in all the examples investigated, to have arisen from the fusion of the two anterior lodicules.

The most complete grass flowers possess two alternating whorls of three stamens. However, this number is known in relatively few genera; more frequently the flowers have fewer stamens. The stamens are free from each other except in a few genera such as *Gigantochloa* and *Oxytenanthera*, where their filaments are united into a tube (Arber, 1934). Rarely the stamens are more than six, as in the genus *Luziola*, where according to Schuster (1910) the extra stamens have arisen by division of the basic six and do not represent extra whorls.

Terminating the floral axis is the gynoecium which is uniloculate and contains a single ovule attached to the posterior wall of the ovary near its base. The ovary is generally accepted to be tricarpellary, and in several genera the ovary is surmounted by three well-developed stigmas. In most other genera there are only two stigmas, but in these the third is usually represented by a stub of tissue which may be more or less well developed. Where there is only one stigma, as in *Zea*, it can be shown that this has arisen from the fusion of two.

The flowers of all genera are basically bisexual, but are frequently unisexual by abortion. In these reduced flowers the organs of the opposite sex are usually present but poorly developed. Neuter flowers, that is, flowers consisting of lodicules only, would be very inconspicuous and appear to have been recorded only for the genus *Trachys* (Pilger, 1940).

Though there is considerable variation in the morphology of flowers in the family Gramineae, most of it may be accounted for by assuming that they have evolved from an *Arundinaria*-type flower whose floral diagram is shown in fig. 1. This is the most complete of known grass flowers, and has a

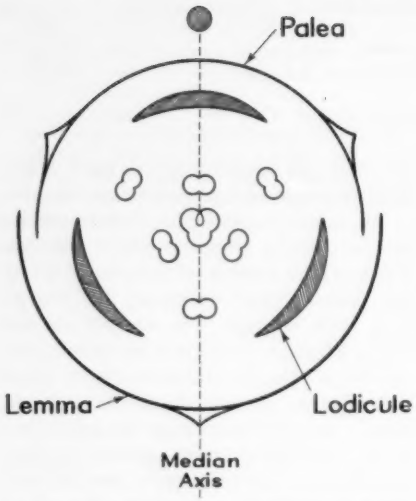


FIG. 1. Floral diagram of *Arundinaria*-type flower.

basic lilifloral structure except in lacking an outer perianth whorl. Evolution from this basic pattern has occurred in three ways, by fusion, suppression, and division of parts.

Amongst present-day grasses there are few genera with flowers possessing fused or divided parts. The majority differ from the basic grass flower in possessing fewer than the maximum possible number of organs. It is only these that will be considered further.

The extent of the variation amongst these reduced flowers is summarized in table 1, in which the different floral types are presented as floral formulae. From these the floral diagrams may easily be reconstructed, as all the formulae refer to bilaterally symmetrical flowers. In preparing the formulae, staminodes and aborted ovaries have been neglected.

Examples of asymmetrical flowers are known, but as they are rare and usually intermixed with symmetrical flowers they are regarded as abnormal and will not be considered further.

In what follows the genera *Ochlandra* and *Anomochloa* are ignored, as neither is closely related to the remainder of the grasses. It has been suggested by Hubbard (1934) that

TABLE 1. *A list of known floral formulae in the Gramineae, restricted to flowers that may be derived by reduction from an* Arundinaria*-type flower*

Floral formula	Representative genus	Authority
$P_3A_{3+3}G(3)$	*Schizostachyum (Arundinaria)*	Arber, 1934
$P_3A_{3+0}G(3)$	*Stipa*	Arber, 1934
$P_3A_{0+0}G(3)$	*Olyra*	Clifford
$P_3A_{3+0}G$ 0	*Olyra*	Clifford
$P_3A_{2+0}G(3)$	*Streptogyna*	Hubbard, 1956
$P_3A_{2+0}G$ 0	*Diandrolyra*	Stapf, 1906
$P_2A_{2+2}G(3)$	*Oryza*	Clifford
$P_2A_{1+2}G(3)$	*Microlaena*	Rossberg, 1934
$P_3A_{1+1}G(3)$	*Woodrowia*	Stapf, 1896
$P_2A_{2+0}G(3)$	*Avena*	Clifford
$P_2A_{2+0}G(3)$	*Eragrostis*	Clifford
$P_2A_{1+0}G(3)$	*Vulpia (Festuca)*	Arber, 1934
$P_2A_{0+1}G(3)$	*Cinna*	Rossberg, 1934
$P_2A_{0+0}G(3)$	*Coix*	Arber, 1934
$P_2A_{2+2}G$ 0	*Zizania*	Weatherwax, 1929
$P_2A_{2+2}G$ 0	*Humbertochloa*	Hubbard, 1939
$P_2A_{2+1}G$ 0	*Zea*	Cutler and Cutler, 1948
$P_2A_{2+0}G$ 0	*Zea*	Clifford
$P_2A_{2+0}G$ 0	*Zea*	Cutler and Cutler, 1948
$P_2A_{0+0}G$ 0	*Trachys*	Pilger, 1940
$P_0A_{2+0}G(3)$	*Nardus*	Hubbard, 1934
$P_0A_{2+0}G(3)$	*Micraira*	Clifford
$P_0A_{1+1}G(3)$	*Anthoxanthum*	Arber, 1934
$P_0A_{1+0}G(3)$	*Imperata*	Haeckel, 1889
$P_0A_{0+0}G(3)$	*Cyphochlaena*	Pilger, 1940
$P_0A_{2+2}G$ 0	*Hydrochloa*	Weatherwax, 1929
$P_0A_{2+0}G$ 0	*Anthephora*	Stapf and Hubbard, 1934
$P_0A_{2+0}G$ 0	*Lophopogon*	Pilger, 1940

Anomochloa may not properly belong to the family Gramineae, which opinion is shared by the writer. The writer would furthermore suggest the genus *Ochlandra* be removed from the family for the following reasons: its lodicules are numerous and spirally arranged instead of three or less in one whorl, it has more than three vascular bundles in the gynoecium, and it has three instead of one vascular bundle in the staminal filaments (Arber, 1934).

ANALYSIS OF FLORAL STRUCTURE

Evolution by reduction from an *Arundinaria*-type flower has often been suggested (Schuster, 1910; Weatherwax, 1929; Rossberg, 1934; Arber, 1934), and the additional

data presented above support this view. The loss of parts during this evolution has not been haphazard, and Weatherwax (1929) has previously drawn attention to the fact that in the course of evolution bilateral symmetry of the flower has been maintained.

Thus, in the course of evolution, organs on a line joining the palea and lemma have been lost individually whereas those not on this axis have been lost in pairs. For example, considering the lodicules only, either the posterior lodicule or the two anterior lodicules may be lost without the resulting flower being asymmetrical, but neither of the anterior lodicules could be lost without introducing asymmetry.

As there must be a number of flowers that may be derived solely by reduction from the *Arundinaria*-type flower, it was thought that a comparison of the known floral types with those theoretically possible would shed light on floral evolution within the family.

The total number obtainable by suppression of parts, whilst still retaining the symmetry required, is easily calculated. There are seven organs or pairs of organs that may be lost without destroying the bilateral symmetry of the flower. The single organs are four in number, the posterior lodicule, anterior stamen of the outer staminal whorl, the posterior stamen of the inner whorl, and the gynoecium; the paired organs are three in number, namely, the anterior lodicules, the posterior stamens of the outer whorl, and the anterior stamens of the inner whorl. These seven organs or pairs of organs can be grouped in 2^7 different ways, but as this includes the possibility of them all being absent or all present the total number of flowers that may be derived is two less than this, namely 126.

Besides knowing the total, it is also important to know the individual numbers of possible flowers with given numbers of parts. These were calculated as follows:

Let a be the number of organs that may occur singly on the median axis of the flower, and b be the number of organs that may occur in pairs on either side of the central axis. Using this notation, in any flower with n parts, a and b must be related in such a way that

$$n = a + 2b$$

For given values of n there will usually be several values of a and b that will satisfy this equation. For example, when $n = 6$ there are the following three pairs:

$$a = 4,\ b = 1;$$
$$a = 2,\ b = 2;$$
$$a = 0,\ b = 3;$$

Furthermore, corresponding to each pair a, b, there may be several combinations of organs. For example, in flowers with $a = 2$, $b = 2$, any two of the four organs on the median axis may be present along with any two of the three pairs of lateral organs. In general, the number of possible flowers to correspond to any pair of values a, b will be given by the product ${}^4C_a \cdot {}^3C_b$.

The number, say N_n, of different possible flowers with n parts is found by summing these products, for example:

$$N_6 = \sum_{b=1}^{3} {}^4C_a \cdot {}^3C_b$$
$$= {}^4C_4 \cdot {}^3C_1 + {}^4C_2 \cdot {}^3C_2 + {}^4C_0 \cdot {}^3C_3$$

With the aid of the above formulae these totals for the different values of n have been calculated and the results are summarized in table 2 along with the numbers of different known floral types with given numbers of parts.

From the table it is clear that the known flowers with given numbers of parts are not a random sample of those possible. A greater proportion of flowers with few parts are known than of flowers with many parts.

TABLE 2. *The numbers of theoretically possible and known flowers with given numbers of organs*

	Number of organs in flower								
	1	2	3	4	5	6	7	8	9
Possible	4	9	16	22	24	22	16	9	4
Known	1	3	4	5	4	5	3	1	1
$\frac{\text{Known}}{\text{Possible}}$ %	25	33	25	23	17	23	19	11	25

TABLE 3. *The theoretically possible and known numbers of flowers with given numbers of lodicules or stamens*

	No. of lodicules or stamens in flower											
	Lodicules				Stamens							
	0	1	2	3	0	1	2	3	4	5	6	
Possible	31	32	32	31	7	16	24	32	24	16	7	
Known	8	0	14	5	4	3	8	6	2	1	3	
Known / Possible	% 26	0	44	16	57	19	33	19	8	6	43	

TABLE 4. *The numbers of theoretically possible and the known flowers of different sex in the Gramineae*

	Male	Female	Bisexual	Neuter
Possible	60	4	59	3
Known	10	3	13	1
Known / Possible %	15	75	20	33

This, it is suggested, results from the loss of organs in whorls rather than as units.

Certain combinations of characters that occur, compared with those possible on the reduction theory proposed, have also been investigated. The various possible floral diagrams containing given numbers of organs were written down by inspection, using the calculated totals for each group to ensure that none were overlooked. With the aid of these floral diagrams comparisons were made of the possible and known numbers for several different character combinations.

One such analysis is summarized in table 3, from which it may be seen that known grass flowers are not merely a random sample of those that are theoretically possible.[1]

The only grass flowers possessing an adaxial lodicule are those with a trimerous perianth. Even in these the posterior lodicule is usually less well developed than the two anterior, suggesting that it is vestigial. Functionally the adaxial lodicule is superfluous, for the two anterior lodicules alone are able to separate the palea and lemma at flowering and in present-day grasses the perianth largely serves this purpose.

Similarly, the known flowers with given numbers of stamens are not a random sample of those possible, and there are proportionately less known flowers with 1, 3, 4, and 5 stamens than there are flowers with 0, 2, or 6 stamens.

Many other analyses are possible but only one other will be considered, that is, the relative frequencies of known and theoretically possible flowers of different sexual status. These are summarized in table 4, from which it is evident that the numbers of flowers possible in the different sexual states are very unequal. There are very few possible female or neuter flowers, whereas there are numerous possible bisexual and male flowers. The known flowers include a greater proportion of female and neuter flowers than either male or bisexual, but little importance can be attached to this as so few female and neuter flowers are involved.

Nevertheless, the data show that the significance of remarks as to the rarity or otherwise of known forms depends in part on the frequency of the possible floral types as well as of those known.

DISCUSSION

The above analyses are only a few of the many possible, but they show that evolution by reduction even from a flower as simple as that of *Arundinaria* can produce many different floral types. If to this number there are added those flowers that could arise by fusion of parts within and between whorls, and those that could arise by the division of existing organs, the total possible number is very much larger.

Yet, as shown above, only about a quarter of the theoretically possible number of flowers is known of those that may be derived by

[1] For example there are no known grass flowers in which the perianth is represented by a single lodicule, except for those in which it has arisen from the fusion of the two abaxial lodicules. The unknown flowers would possess the single adaxial lodicule, and their absence accounts for about a quarter of the unknown but theoretically possible floral types.

reduction. The reasons for this are difficult to determine and could be due to any one of several causes operating alone or in combination.

It has been assumed above that table 1 includes all or most of the different grass flowers that are known, but although a good deal of literature has been consulted, some published floral types may have been overlooked. Besides these, there may be some undescribed flowers which have not yet been discovered, but it is thought that these are few.

Another reason for the absence of certain of the theoretically possible flowers is that they may have occurred in the past but have been lost through the species involved becoming extinct. The fossil record provides no useful information for, although fossil grasses are known from as early as the Cretaceous period (Arnold, 1947), no flowers appear to have been preserved.

Some of the unknown flowers may yet evolve, but without knowing more of the selective forces to which the plants will be subjected nothing can be predicted about these.

Finally, it may be that those flowers not found involve incompatible arrangements of organs. Some support for this view comes from the presence of the asymmetrical aberrant flowers mentioned above. That such flowers are possible is shown by their occurrence, but that such combinations involve exceptional development steps is shown by their infrequency. That is, development has been canalized into the forms commonly met. Why these forms should have been at a selective advantage in the evolution of the family is again a difficult question.

The reduction or even the total loss of the perianth would appear to be an adaption for wind pollination. Today, apart from *Pariana*, which may be regularly insect pollinated (Davis and Richards, 1933), the grasses are generally cleistogamous or wind-pollinated.

If it is assumed that the primitive grasses belonged to the wet tropics and occupied forest or near-forest habitats, then they were probably insect-pollinated and possessed a perianth. Most genera with three lodicules are today confined to areas with warm to hot climates, and the genus *Streptochaeta* which belongs to this group has the best developed of all grass perianths.

On expanding from their tropical forest habitats onto the plains of the world a change to wind pollination might well be an advantage, and so the reduction of the perianth in more advanced grass flowers is easily understood.

There would not be a similar advantage in the loss of stamens. Yet few modern grasses possess their full complement. Compensation for the loss of stamens may be reflected in the development of the paniculate inflorescence with its numerous flowers, and also in the production of numerous male flowers in the inflorescences of more specialized grasses such as those belonging to the subfamily Panicoideae.

A similar compensation might be seen in the strong development of vegetative reproduction within the family and the numerous apomictic and cleistogamic species that are known may reflect an offset to the risks associated with wind pollination.

Summary

It has been assumed that the *Arundinaria*-type flower is primitive in the family Gramineae. On the basis of this assumption the number of bilaterally symmetrical flowers that may be derived solely by reduction from this flower has been calculated and compared with the known number. Only about a quarter of the theoretically possible flowers are known, and five reasons are given to account for those that are missing. They may yet be discovered, or may have been discovered but overlooked by the writer; they may have existed in the geological past or may yet evolve; finally, they may be developmentally impossible through involving incompatible arrangements of organs.

The primitive grass flower is assumed to have possessed a well-developed perianth and to have been insect-pollinated. In the

course of evolution it has lost most of its perianth and become wind-pollinated.

It is suggested that the transition from insect to wind pollination, and the reduction in stamen number within the flower in the course of evolution, has been accompanied by the development of the paniculate inflorescence, the marked tendency for vegetative reproduction, and the strong development of cleistogamy and apomixis in the family.

Acknowledgments

The writer has pleasure in recording his thanks to Drs. J. E. C. Aberdeen, D. W. Goodall, and G. R. Morris for their helpful discussions during the preparation of this paper and to Mr. E. E. Savage of the Department of Geography for the preparation of fig. 1.

Literature Cited

Arber, A. 1934. The Gramineae, a Study of Cereal, Bamboo and Grass. Cambridge, Univ. Press.

Arnold, C. A. 1947. An Introduction to Paleobotany. New York. McGraw-Hill.

Cutler, H. C., and M. C. Cutler. 1948. Studies on the structure of the maize plant. Ann. Mo. Bot. Garden, **35**: 301–316.

Davis, J. A. W., and P. W. Richards. 1933. The vegetation of Moraballi Creek, British Guiana. Pt. 1. J. Ecol., **21**: 350–384.

Haeckel, E. 1887. Gramineae. Die natürlichen Pflanzenfamilien Teil 2, Abt. 2. Leipzig.

——. 1889. Andropogoneae (Gramineae), Monogr. Phan. vol. 6. Paris.

Hubbard, C. E. 1934. Gramineae. *In* "Families of Flowering Plants." Vol. 2. Monocotyledons, J. Hutchinson. London, Macmillan.

——. 1939. Hook. Icon. Plant. t. 3387.

——. 1956. Hook. Icon. Plant. t. 3572.

Lindley, J. 1846. The Vegetable Kingdom. London. Parbury and Evans.

Pilger, R. 1940. Gramineae. Die natürlichen Pflanzenfamilien 14e. Leipzig.

Rossberg, G. 1935. Beitrage zur Morphologie des Grasährchens. Inaug. Diss., Frankenburg.

Schuster, J. 1910. Ueber die Morphologie der Grasblute. Flora, **100**: 213–266.

Stapf, O. 1896. Hook. Icon. Plant. t. 2447.

——. 1906. Kew Bull., **1906**: 204.

—— and Hubbard, C. E. 1934. Flora of Tropical Africa, **9**: 933. Ashford, L. Reeve.

Stebbins, G. L. 1951. Natural Selection and Differentiation of Angiosperm Families. Evolution, **5**: 299–324.

Weatherwax, P. 1929. The Morphology of the Spikelets of Six Genera of Oryzeae. Amer. J. Bot., **16**: 547–555.

THE EVOLUTIONARY STATUS OF *DROSOPHILA SERRATA*

THEODOSIUS DOBZHANSKY AND WHARTON B. MATHER
Departments of Zoology, Columbia University, New York City, and University of Queensland, Brisbane, Australia

Received March 1, 1961

One of the peculiarities of the genus *Drosophila* is the abundance in it of pairs, and of groups of several, sibling species. Siblings are species morphologically similar enough to be practically indistinguishable by inspection, and yet having attained complete or almost complete reproductive isolation. It is tempting to speculate that the evolution of morphological features has reached, in this genus, a high degree of perfection, and that the adaptive evolution is directed largely in physiological channels (Dobzhansky, 1956). Species being so often morphologically close, it is not surprising that geographic races of *Drosophila* species are as a general rule morphologically indistinguishable (cf. Patterson and Stone, 1952; see, however, Stalker and Carson, 1947, and Prevosti, 1954, 1955). In fact, the taxonomic category of subspecies has been seldom made use of in *Drosophila*, at least as compared to other groups of animals the systematics of which has achieved an "advanced" stage. In *Drosophila*, geographic populations, or races, of a species differ more often in such "recondite" traits as the gene arrangements in their chromosomes, which must, however, be regarded as connoting physiological differentiations. Instances of conspicuous variation in visible traits are, to be sure, known among *Drosophila*, but they usually represent intrapopulational balanced polymorphisms. The best studied case of this sort is the variation of the coloration of the abdomen in *Drosophila polymorpha* (da Cunha, 1949); the case of *Drosophila montium* is alleged to be similar (Freire-Maia, 1949), but the published data are inconclusive.

Drosophila serrata Malloch (1927) is a species belonging to the *melanogaster* species group of the subgenus *Sophophora*, described from a locality (Eidsvold) in Queensland, Australia. A redescription, in the form now accepted in *Drosophila* systematics, can be found in Mather, 1955. We have collected strains belonging to this species from several localities in eastern Queensland, in New South Wales, in New Guinea, and New Britain. Two morphologically distinguishable subspecies can be recognized among these strains. Incipient reproductive isolation, particularly sexual isolation and some hybrid sterility, is also observed among these strains. Interestingly enough, the limits of the morphological subspecies do not coincide, however, with those of the reproductively isolated groups of populations. Observations and experiments bearing on the evolutionary status of this remarkable species are reported below.

GEOGRAPHIC DISTRIBUTION

We have in our laboratories living strains of *Drosophila serrata* descended from mass-cultured founders collected in the localities shown in fig. 1. In more detail, these localities are: Bulahdelah, some 130 miles north of Sydney, New South Wales (this population is referred to below as "Sydney"); Moggill Farm, Brisbane, Queensland (referred to as "Brisbane"); Mundubbera, Queensland; Burnett River, Queensland; Proserpine, Queensland; Crystal Cascade, near Cairns, Queensland (referred to as "Cairns"); Bisianumu Plantation, Sogeri Plateau, north of Port Moresby, Territory of Papua (referred to as "Moresby"); Bulolo Gorge, between Bulolo and Wau, Territory of New Guinea (referred to as "Bulolo"); Keravat, near Rabaul, New Britain (referred to as "Rabaul"). The species has also been found at Gordonvale, near Cairns, and at Lake Barrine, Atherton Plateau, Queensland; in the valley of Musgrave River, Territory of Papua; for more

FIG. 1. Geographic origin of the strains of *Drosophila serrata* which served as material for the present investigation.

localities in eastern Queensland, see Mather, 1955. It may be worth while to note that the species has *not* been encountered in samples of *Drosophila* from the vicinities of Sydney; in the valley of Brown River, near Port Moresby; near Goroka and on the road to Daulo Pass, eastern Highlands of New Guinea; and only a few specimens were encountered in the vicinity of Lae, New Guinea, and none of them produced viable progenies. It remains to be discovered whether our species extends its distribution to central and western Queensland and the territories beyond.

MORPHOLOGY

One of the traits which permits division of the strains at our disposal into two clear-cut subspecies, the southern one, *Drosophila serrata serrata*, and the northern one, *Drosophila serrata birchii*, is found in the male genitalia. The difference can easily be seen in both living and dried male specimens under a moderate to high magnification of a dissecting microscope. The strains of *serrata serrata* (Sydney, Brisbane, Mundubbera, Burnett River, and Proserpine) all have two pairs of strong bristles on the genital arch (fig. 2). Males from the Greenslope, Queensland, strain used by Mather also had two pairs of bristles (fig. 9A, in Mather, 1955). The males of *serrata birchii* (Cairns, Moresby, Bulolo, and Rabaul) have three pairs of bristles on the genital arches (fig. 3). At least 20 males have been examined from every strain, without finding a single exception to the rule.

The strains fall into the same two groups also on the basis of a quite different trait—the coloration of the dorsal part of the abdomen in the females. This trait is easily visible in females several days old after the hatching from pupae, but not in young females which are not fully hardened. The subspecies *serrata serrata* has females with dark abdomens. The dark brown bands along the posterior borders of the abdominal

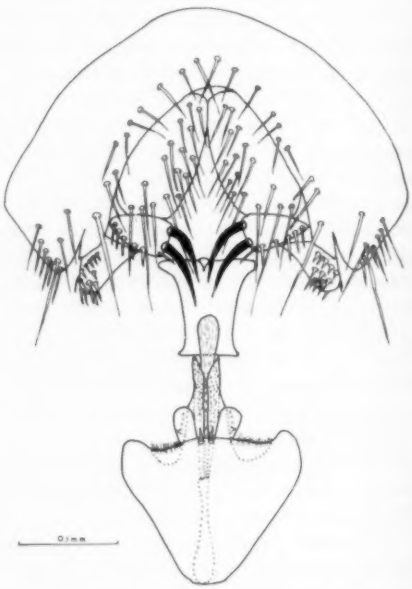


FIG. 2. Male genitalia of *Drosophila serrata serrata* Malloch.

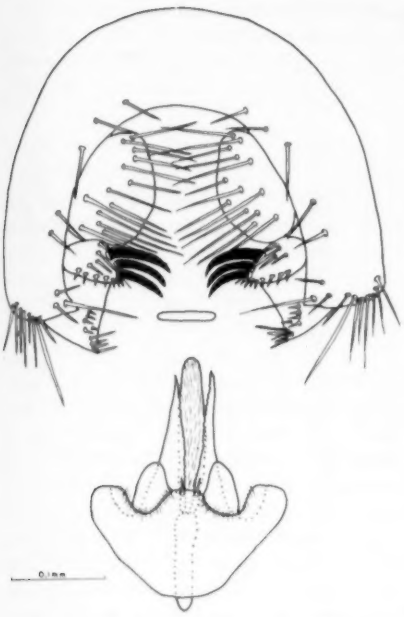


FIG. 3. Male genitalia of *Drosophila serrata birchii* subsp. nov.

tergites are expanded laterally towards the antero-lateral angles of the tergites. The outlines of these bands are not sharp, making the whole abdomen seem fuscous-brown in color; the lighter parts are the middles of the anterior parts and the posterior lateral angles of the tergites. Females of *serrata birchii* have lighter abdomens. In most females, the dark bands are found only on the posterior margins of the 2nd to 5th tergites. These bands are narrowing, rather than expanding laterally, as a rule not reaching the lateral margins. They are more or less sharply delineated, dark brown to black, but leaving most of the tergite brownish yellow.

The coloration of the abdomen varies within subspecies, as well as between the subspecies, and within as well as between strains. The Brisbane, Cairns, and Moresby strains contain some relatively dark and relatively light individuals, with a distinct suggestion of a bimodality. We have raised separately progenies of darker and of lighter females, and they proved to be darker and lighter on the average respectively. An attempt to divide the flies into discrete classes was, however, felt to be giving untrustworthy results. Moreover, since the males show no bimodality in the abdominal coloration, the characteristics of one of the parents in every cross are uncertain, complicating the genetic analysis.

As to the interstrain but intrasubspecific variation, the situation can be described as suggesting the existence of south–north gradients in the coloration of the female abdomen within each subspecies. In either subspecies, the more southern strains tend to be darker than the more northern ones. The darkest *serrata serrata* are the Sydney strain, while Brisbane, Mundubbera, Burnett River, and Proserpine are lighter. The lightest strain of *serrata birchii* is Rabaul, and the darkest either Moresby or Cairns, Bulolo being intermediate. It should be made clear that these interstrain variations do not erase the differences between the two subspecies. We can recognize the subspecies of a single living female by the color of her abdomen. A group of females from Rabaul is also recognizable as being lighter on the average than a group of Moresby or Cairns females. Some individuals seem, however, intermediate, and would be misclassified if examined singly.

There are indications of differences between the two subspecies also in other characters. Males of *serrata birchii* are generally lighter in coloration than *serrata serrata*, but the difference is not reliable. The females of the former tend to be lighter all over than in the latter, but only the abdominal color is diagnostic. Figs. 2 and 3 suggest differences in the penis and the gonapophyses, those of *serrata birchii* being longer than in *serrata serrata*. How reliable these characters are for classification is uncertain.

Malloch's type locality of *Drosophila serrata* being very close to Mundubbera and Burnett River, it seems safe to conclude that our southern subspecies is the typical one.

TABLE 1. *Sexual preferences among strains of* Drosophila serrata
S = Drosophila serrata serrata, *B* = Drosophila serrata birchii

Females	Males	Females tested	Number inseminated	Per cent inseminated
Sydney (S)	Sydney (S)	19	18	95
"	Brisbane (S)	19	19	100
"	Mundubbera (S)	9	9	100
"	Burnett River (S)	10	10	100
"	Cairns (B)	38	0	0
"	Moresby (B)	49	43	88
"	Bulolo (B)	24	0	0
"	Rabaul (B)	20	0	0
Brisbane (S)	Brisbane (S)	20	18	90
"	Sydney (S)	26	24	92
"	Mundubbera (S)	9	9	100
"	Burnett River (S)	8	8	100
"	Proserpine (S)	30	28	93
"	Cairns (B)	42	1	2
"	Moresby (B)	71	44	62
"	Bulolo (B)	22	1	5
"	Rabaul (B)	25	0	0
Mundubbera (S)	Bulolo (B)	18	0	0
Cairns (B)	Cairns (B)	18	18	100
"	Sydney (S)	25	0	0
"	Brisbane (S)	35	1	3
"	Mundubbera (S)	19	1	5
"	Burnett River (S)	14	0	0
"	Proserpine (S)	19	0	0
"	Moresby (B)	104	25	24
"	Bulolo (B)	34	26	76
"	Rabaul (B)	120	59	49
Moresby (B)	Sydney (S)	33	12	36
"	Brisbane (S)	22	17	77
"	Cairns (B)	40	16	40
"	Bulolo (B)	35	2	6
"	Rabaul (B)	36	10	28
Bulolo (B)	Sydney (S)	9	0	0
"	Brisbane (S)	22	0	0
"	Mundubbera (S)	26	0	0
"	Cairns (B)	23	23	100
"	Moresby (B)	43	2	5
"	Rabaul (B)	23	22	96
Rabaul (B)	Rabaul (B)	20	18	90
"	Sydney (S)	23	1	4
"	Brisbane (S)	13	0	0
"	Cairns (B)	42	31	74
"	Moresby (B)	43	14	33
"	Bulolo (B)	23	21	91

A formal description of the northern subspecies can be given as follows:

Drosophila serrata subspecies *birchii* Dobzhansky and Mather

Differs from *Drosophila serrata serrata* Malloch (1927) in having three, instead of two, pairs of strong bristles on the genital arch in the males. Females of *birchii* have more lightly colored abdomens, the dark bands on the posterior margins of the abdominal tergites being sharply delimited anteriorly, and narrowing, instead of expanding, towards the lateral margins of the tergites.

Type Locality—Rain forest of the Crystal Cascade Park, near Cairns, Queensland, Australia. The type deposited in the Australia Museum, Sydney, Australia.

Sexual Isolation

First attempts at crossing the two subspecies having failed, we have made a systematic study of the sexual isolation between them. Groups of 10 virgin females from various strains were placed in ordinary culture bottles with about 10 males of the same or of a different strain, and kept in a lighted constant-temperature room at 25° C. In about 5 days, the females and males were transferred, without etherization, to fresh culture bottles. About 5 days or a week later, the surviving females were dissected, and their spermathecae were examined under a microscope for the presence or absence of sperm. The data obtained are summarized in table 1. It can be seen that, with most combinations of strains, the results are practically of all-or-none kind, almost all females being either inseminated or almost all left virgin. This fact seemed to us to make unnecessary for our purposes to use the more cumbersome "male-choice" method, i.e., exposing two kinds of females to one kind of males.

Intercrosses of the strains of *serrata serrata* occur quite easily. Every one of the 9 intercrosses of this sort recorded in table 1 gives 90 to 100 per cent of the females inseminated. In the subspecies *serrata birchii* the situation is more complex. Insemination of 90 or more per cent of the females has been obtained only when females and males came from the same strain, and also in the crosses Bulolo♀ × Cairns♂, Bulolo♀ × Rabaul♂, and Rabaul♀ × Bulolo♂. Most peculiar is the behavior of the Moresby strain; although according to its morphology it belongs unambiguously to the subspecies *serrata birchii*, it intercrosses actually more easily with some *serrata serrata* strains (Sydney♀ × Moresby♂, Brisbane♀ × Moresby♂, Moresby ♀ × Brisbane♂) than to other strains of *serrata birchii* Cairns♂, Moresby♀ × Bulolo♂, Moresby♀ × Rabaul♂, Bulolo♀ × Moresby♂, and Rabaul♀ × Moresby♂).

Disregarding the peculiar behavior of the Moresby strain, the crosses of *serrata serrata* with *serrata birchii* result either in a lack of insemination or in insemination of only a scattering of females.

Hybrid Sterility

Many of the culture bottles in which the females of one strain were being tested for cross-insemination by males of other strains produced hybrid progenies. All the hybrid progenies were routinely tested for fertility of the F_1 hybrid flies. Attention was paid especially to the progenies of the crosses which, as shown in table 1, result in few cross-inseminations. The results are clear and unambiguous. Except where one of the parents of the cross came from the Rabaul strain, the F_1 hybrids are fertile, and F_2 progenies are produced easily and abundantly. The hybrids having Rabaul as one of the parents produce, on the contrary, no F_2 progenies. It is easily shown that the difficulty is due to sterility of the hybrid males; the hybrid females are fertile, and produce back-cross progenies with other males. Most remarkable of all, the hybrids of Rabaul with other strains of *serrata birchii* (Cairns, Moresby, and Bulolo) are just as sterile as are the male hybrids with *serrata serrata* (Rabaul♀ × Sydney♂, table 1).

Discussion

The data presented in the foregoing pages show clearly enough that evolutionary phenomena of considerable interest can be observed in *Drosophila serrata*. It is especially evident that much more information is needed for a satisfactory understanding of these phenomena. Had our material been restricted to strains from the continent of Australia, the subspecies *serrata serrata* and *serrata birchii* would appear clearly demarcated, and would seem to be standing on the threshold of full species status. Even with these continental strains, it remains, however, unknown whether the distribution areas of the subspecies do or do not overlap,

and if they do whether hybrids or intermediates occur in nature. The only living strain of *serrata birchii* from Queensland is that from Cairns; other strains from the same region (Gordonvale, Lake Barrine) were recorded to have the same abdominal coloration in the females as in the Cairns strain, but they were, unfortunately, discarded before other tests could have been made. The nearest known locality to the south is Proserpine; the Proserpine strain, derived from a few flies hatched from garden fruit collected for another purpose, belongs clearly to *serrata serrata*. Further material from the Cairns-Proserpine region, and from the territory lying farther inland from there, is most desirable.

Crossing the Torres Strait, which separates Australia from New Guinea, discloses a very complex situation. The Moresby strain, derived from the mountains north of Port Moresby, is morphologically very similar to *serrata birchii* from Cairns. Yet the sexual preferences of the Moresby flies are unlike either those of other strains of *serrata birchii*, or of *serrata serrata*. It is to some extent sexually isolated from both, but completely isolated from neither. Crossing the Owen Stanley Mountains, we find on the northern slope of this range, at Bulolo, a population of *serrata birchii* which is actually more like that from Cairns than that from the geographically much closer Moresby. We do not know whether the species inhabits the upper reaches of the Owen Stanley; as stated above, we failed to find *D. serrata* in the highlands in the Goroka region. Moresby and Bulolo may actually be more strongly isolated than their geographic proximity would suggest.

Crossing the Vitiaz Strait, between New Guinea and New Britain, brings a new complication. The Rabaul strain is morphologically *serrata birchii*, and its sexual preferences are accordingly for other strains of the same subspecies (except, as stated above, the Moresby strain), and against *serrata serrata*. And yet, Rabaul produces sterile male hybrids with all other strains at our disposal. It would be most interesting to discover the behavior of the populations from other islands of the Bismarck Archipelago, and from the Solomons, if the species lives there.

If we were to take hybrid sterility as the criterion of subspecific differentiation, the limits of the subspecies would be different from those drawn on the basis of morphology. We would place Rabaul in one subspecies, and all other strains in the second. If sexual preferences were used as the criterion, the division would be still different, at least tripartite rather than bipartite (compare the behavior of the Moresby strain). This is the sort of situation which has led some authors to declare the subspecies category invalid. We find it, however, convenient to have short names to refer to the easily recognizable groups of populations. Now, a certain arbitrariness of the intraspecific divisions is not an accident but a reflection of the biologically meaningful fact that populations, races, or subspecies are genetically open systems, contrasting with species which are genetically closed systems. The genetic closure is, indeed, the essence of speciation.

The incipient speciation in *D. serrata* may be compared with that in the tropical American *D. paulistorum* (Dobzhansky and Spassky, 1959; Ehrman, 1960). In the former the subspecies have become morphologically distinguishable, while in the latter they are not. In both species there appeared reproductive isolation between the subspecies; yet in *D. paulistorum* the isolation has attained the stage when some of the incipient species coexist sympatrically without mixing. One wonders whether these differences may be related to *D. paulistorum* being an essentially continental species, while *D. serrata* crosses some important water barriers. Further study of *D. serrata*, particularly a comparison of the situation on the continent of Australia with that on the adjacent islands, may shed new light on this situation.

Acknowledgments

The travel and work of one of us (Th. D.) in Australia and New Guinea was made pos-

sible by the tenure of the Fulbright and J. S. Guggenheim Foundations fellowships at the Department of Zoology, University of Sydney. We are obliged to a number of colleagues, particularly to Professor Charles Birch of Sydney, Dr. J. J. H. Szent-Ivany of Port Moresby, Mr. A. Catley of Lae, and Mr. G. S. Dunn of Keravat for their assistance and hospitality. The work in Australia was assisted by Dr. Patricia Elliott and Mr. J. H. Hawes, and in New York by Mr. Boris Spassky.

Summary

Strains of *Drosophila serrata* have been collected and studied from Australia (coastal Queensland and New South Wales), New Guinea, and New Britain. Two subspecies, *serrata serrata* and *serrata birchii* nova, have been distinguished on the basis of their external appearance and genital structures. A strong sexual isolation is observed between some of the strains, but this isolation does not coincide with the limits of the morphological subspecies. The behavior of a population from the mountains north of Port Moresby (Territory of Papua) is particularly interesting—this population shows pronounced but incomplete isolation both from *serrata serrata* and *serrata birchii*, although morphologically it belongs to the latter. The population of Rabaul (New Britain) is morphologically also *serrata birchii*, but it produces sterile male hybrids with all strains at our disposal, of *serrata serrata* as well as *serrata birchii*.

Literature Cited

daCunha, A. B. 1949. Genetic analysis of polymorphism of color patterns in *Drosophila polymorpha*. Evolution, **3**: 239–251.

Dobzhansky, T. 1956. What is an adaptive trait? Amer. Nat., **90**: 337–347.

—— and B. Spassky. 1959. *Drosophila paulistorum*, a cluster of species in statu nascendi. Proc. Nat. Acad. Sci., **45**: 419–428.

Ehrman, L. 1960. The genetics of hybrid sterility in *Drosophila paulistorum*. Evolution, **14**: 212–223.

Freire-Maia, N. 1949. Balanced polymorphism in *Drosophila montium*. Evolution, **3**: 98.

Malloch, J. R. 1927. Notes on Australian Diptera. X. Proc. Linnean Soc. N. S. Wales, **52**, Pt. II: 1–16.

Mather, W. B. 1955. The genus *Drosophila* (Diptera) in eastern Queensland. I. Taxonomy. Austr. Jour. Zool., **3**: 545–582.

Patterson, J. T., and W. S. Stone. 1952. Evolution in the genus *Drosophila*. Macmillan, New York.

Prevosti, A. 1954. Variacion geografica de varios caracteres cuantitativos en poblaciones catalanas de *Drosophila subobscura*. Genet. Iberica, **6**: 33–68.

——. 1955. Variacion geografica de caracteres cuantitativos en poblaciones britanicas de *Drosophila subobscura*. Genet. Iberica, **7**: 3–44.

Stalker, H. D., and H. L. Carson. 1947. Morphological evolution in natural populations of *Drosophila robusta*. Evolution, **1**: 237–248.

EVOLUTION IN A GROUP OF INDO-AUSTRALIAN *EUMENES* (HYMENOPTERA, EUMENIDAE)

J. VAN DER VECHT
Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands

Received March 3, 1961

The Indo-Australian Archipelago is one of the most interesting parts of the world from the viewpoint of biogeography. Flora and fauna differ more or less from island to island, the most conspicuous faunal differences occurring on islands separated by the well-known lines of Wallace and of Weber. These differences are partly determined by factors that are the same for all organisms, such as the geological history of the islands, their size and their climatic and soil conditions, and partly by the specific properties of the organisms, such as their dispersal faculties and ecological requirements and tolerances. As regards the geological history, the relatively stable areas of the Sunda shelf and the Sahul shelf, which formed continuous land masses in the Pleistocene, differ considerably from the intermediate unstable area (Rensch, 1936; Mayr, 1944a; Darlington, 1957).

It is accepted by many authors that the land fauna of these islands is the product of successive invasions from the Asiatic continent, which have penetrated southward and eastward at different times and over varying distances. As the descendants of the invaders became isolated from the original stock, they evolved independently, thereby diverging more and more in the course of the time. In analyzing the differences existing at present, some recent authors have made the error of regarding the degree of divergence as being directly proportional to the length of time elapsed since the beginning of the isolation. They have overlooked the fact that evolutionary rates may be strongly influenced by initial differences in genetic constitution as well as by differences in environmental selective pressure. Within a given period of time, the greatest evolutionary changes may be expected from populations which result from small numbers of founders and which have become adapted to rather different ecological conditions (Mayr, 1954).

It is thus understandable that the islands west of Wallace's line, which are close to the mainland and were still connected with it in the Pleistocene, possess a fauna very similar to that of the Asiatic continent, whereas the more distant Australian region, which includes New Guinea and the neighboring islands and which has been more or less isolated for a very long time, has a widely different fauna containing many endemic species or higher categories.

With these considerations in mind, a study has been made of the taxonomy and distribution of a group of wasps of the genus *Eumenes* Latreille (van der Vecht, 1959). These are relatively large wasps, 2–3 cm long, which build nests of clay and store the cells with lepidopterous larvae. These wasps appear to be particularly suitable for this type of investigation. They occur throughout the Archipelago, having spread as far as the Solomon Islands and Northern Australia, and their geological age must be considerable, for the populations inhabiting the various islands have evolved sufficiently to develop marked differences in structure as well as in color.

DISTRIBUTION OF SPECIES OF *EUMENES ARCUATUS* GROUP

The species of this group can be divided into two subgroups, distinguished by some morphological characters, among which the shape of the volsella of the male genitalia appears to be of particular importance. The apex of the digitus volsellaris is long and thin in the subgroup of *Eumenes curvatus* (figs. 1a–1c) and in the rather aberrant *E. incola* (fig. 1d), shorter and approximately

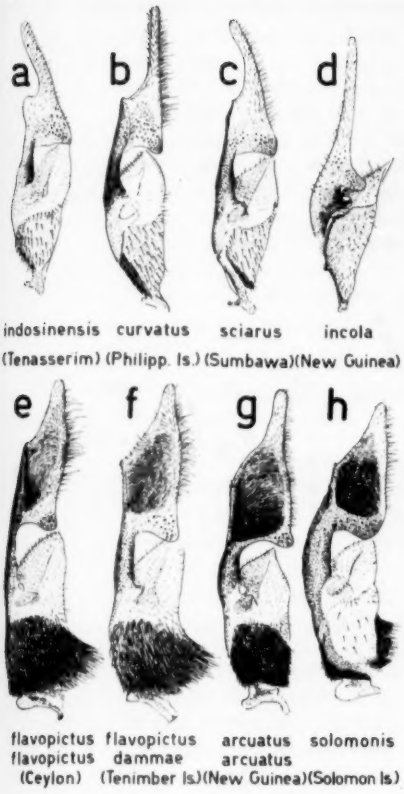


FIG. 1. Volsella of male genitalia in some *Eumenes* species. Top row: subgroup of *E. curvatus* (a–c), and *E. incola* (d); bottom row: subgroup of *E. arcuatus*.

triangular in the species of the subgroup of *E. arcuatus* (figs. 1e–1h).

The present pattern of distribution of these wasps seems best explained by regarding it as the product of two successive invasions of Asiatic origin.

The earlier invaders have reached Australia and New Guinea, where they are now represented by *E. incola* (fig. 2), apparently a forest inhabitant. This species is so aberrant in various respects that one may hesitate to regard it as originating from the same invasion wave which in the western part of the archipelago is represented by the *curvatus* subgroup. Yet we must take into account that this Australian form has probably resulted from a small number of founders and that it may have evolved under conditions rather different from those occurring elsewhere. *E. incola* is a polytypic species, composed of at least six subspecies showing various degrees of reduction of a color pattern which is very similar to the "*arcuatus* pattern" shown in the top row of fig. 4. The *curvatus* subgroup consists of at least eight closely allied, allopatric species which are all black, as a rule with dark wings; only the Celebes species *E. zamenes* van der Vecht has the wings partly yellow.[1] This subgroup may be regarded as a superspecies, evolved from a single species by isolation of several local populations. In continental Southeast Asia and on the islands of the Sunda shelf these wasps are extremely rare inhabitants of primary forests, but in the islands fringing the former Sunda continent (the area east of the broken line in fig. 2) they appear to be fairly common. In the Philippines *E. curvatus* even takes the place of *E. flavopictus* on the Sunda shelf as the common species of the cultivated lowlands. It seems reasonable to explain this difference by supposing that there has been less competitive pressure in the marginal islands.

So far no traces of the earlier invasion have been found in the Moluccas and in the eastern part of the Sunda arc (Timor and Tenimber Islands).

The elements considered to be descendants of a later wave of immigrants from the mainland form the subgroup of *Eumenes arcuatus*, which consists of at least eight structurally different species (figs. 1 and 5). Two of these, *E. flavopictus* and *E. arcuatus*, are polytypic, widespread, and evidently closely related. *E. flavopictus* inhabits tropical continental Asia and the islands of the Sunda shelf, extending eastward along the Sunda arc as far as the Tenimber and probably also the Kei Islands. In all

[1] The association of *E. zamenes* with the *curvatus* group, still regarded as doubtful in 1959, has since been established by the study of a male in the collection of the Museum at Madrid.

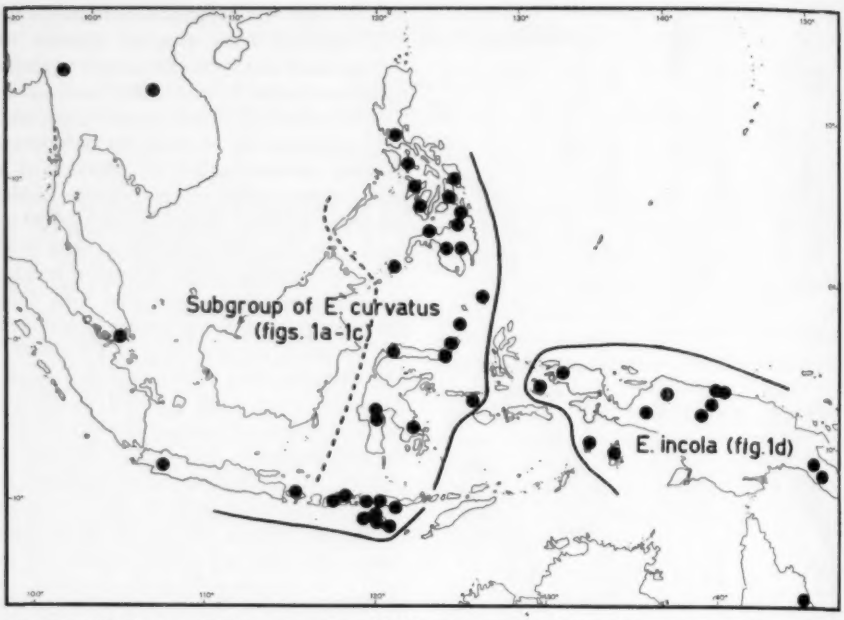


FIG. 2. Distribution of *Eumenes* species with elongate volsellar digitus (figs. 1a–1d). The species of the subgroup of *E. curvatus* are very rare on the Sunda shelf (continental area in the Pleistocene glacial periods), but fairly common in the fringing islands, east of the broken line.

the populations inhabiting this extensive area the base of the volsella is densely covered with long hairs (figs. 1e and 1f). *E. arcuatus* differs from *E. flavopictus* in the shape of the clypeus, which is slightly more emarginate anteriorly, and in the shorter pubescence of the volsellar base (fig. 1g). It occurs in Northern Australia, New Guinea, and in some neighboring islands, including the Moluccas. Both these species are common inhabitants of the cultivated lowlands and lower hills, also occurring in light forests. They are evidently less specialized as regards their ecological requirements than *E. incola* and most species of the *curvatus* subgroup, but unfortunately practically nothing is known about the bionomics of these wasps.

Besides these two polytypic species, the subgroup embraces six monotypic species: four closely allied endemic inhabitants of Celebes and the neighboring islands, and two species occurring in the Solomon Islands. The morphological characters of these isolated species are more or less aberrant; yet it appears that they are more closely related to *E. arcuatus* than to *E. flavopictus*. The Celebes species *E. fulvipennis* is a common lowland species, but its relatives inhabiting Mindanao and some of the Southern Moluccas appear to be rare. Here again differences in competitive pressure may be held responsible. The peculiar shape of the volsella in one of the Solomon Islands species is shown in fig. 1h.

In the further discussion special attention will be given to the geographic variation in the two polytypic species, *E. flavopictus* and *E. arcuatus*. Both these species show little variation in structural details throughout their range, but insular populations may differ very strikingly in color pattern. Each species consists of a "central population" inhabiting an extensive area (therefore also called "mainland population"), and a number of peripheral populations occurring in

the adjacent islands. The central population of *E. flavopictus* is spread from India to Formosa and Borneo; it has the typical *arcuatus* pattern (fig. 4, top row) throughout this extensive area, showing only some clinal reduction of the extent of the yellow markings in the direction from west to east. It is considerably more homogeneous than would appear from a study by Zimmermann (1931), whose locality records have proved to be partly incorrect. The central population of *E. arcuatus* inhabits Northern Australia, New Guinea, the Aru Islands, and probably also the Kei Islands.

Similarity of Central Populations of Sunda and Sahul Shelf

It is of particular interest that the central populations of *E. flavopictus* and *E. arcuatus*, although living far apart, have almost exactly the same color pattern. The resemblance is so striking that it seems very unlikely that the pattern evolved independently in these widespread populations. On the other hand, if one assumes it to be inherited from a common ancestor, the question arises as to why it has remained so constant during a long period of time. This seems contrary to the general rule that color is more variable than structure.

The answer to this question was found when the color pattern of these wasps was compared with that of some similar-looking wasps of the genera *Pareumenes* and *Pseumenes*. For although strongly different in structural characters, these wasps show a remarkable parallel variation in color through much of the range of the *Eumenes* species (van der Vecht, 1937). In these wasps, too, the populations of the Sunda and Sahul shelf, although specifically different, are very similar, whereas two Java forms and at least one form of the Lesser Sunda Islands run exactly parallel with *Eumenes flavopictus* in the progressive reduction of the yellow markings (fig. 3). Evidently the color pattern is influenced by environmental factors, which must be locally the same for these three rather different insects. It seems therefore reasonable to assume that in these

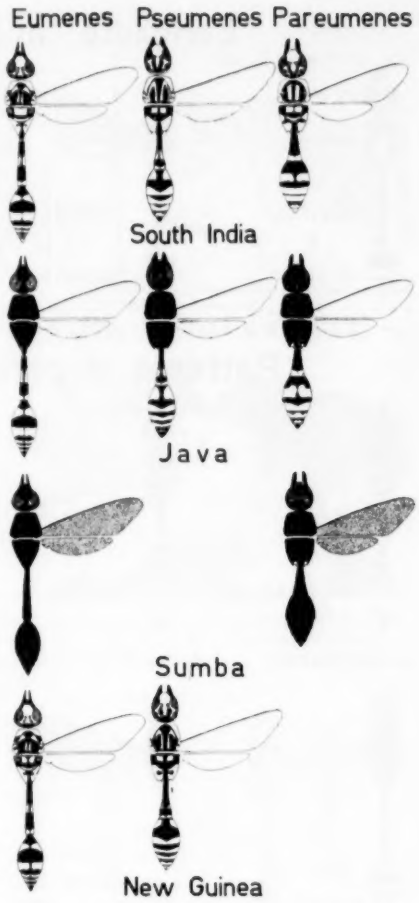


Fig. 3. Parallel variation of color pattern in three genera of Eumenidae.

wasps the color pattern is an allaesthetic character (see Cott, 1954) with a highly efficient warning capacity, these wasps being members of a "Müllerian mimicry" series (see de Ruiter, 1959). Evidently the ancestral pattern has survived wherever environmental conditions have been relatively stable for a long time.

In this connection it is of interest that also the Timor form of *E. flavopictus* has the complete *arcuatus* pattern, particularly since so far *Pareumenes* or *Pseumenes* have not been found in this island. The wasp

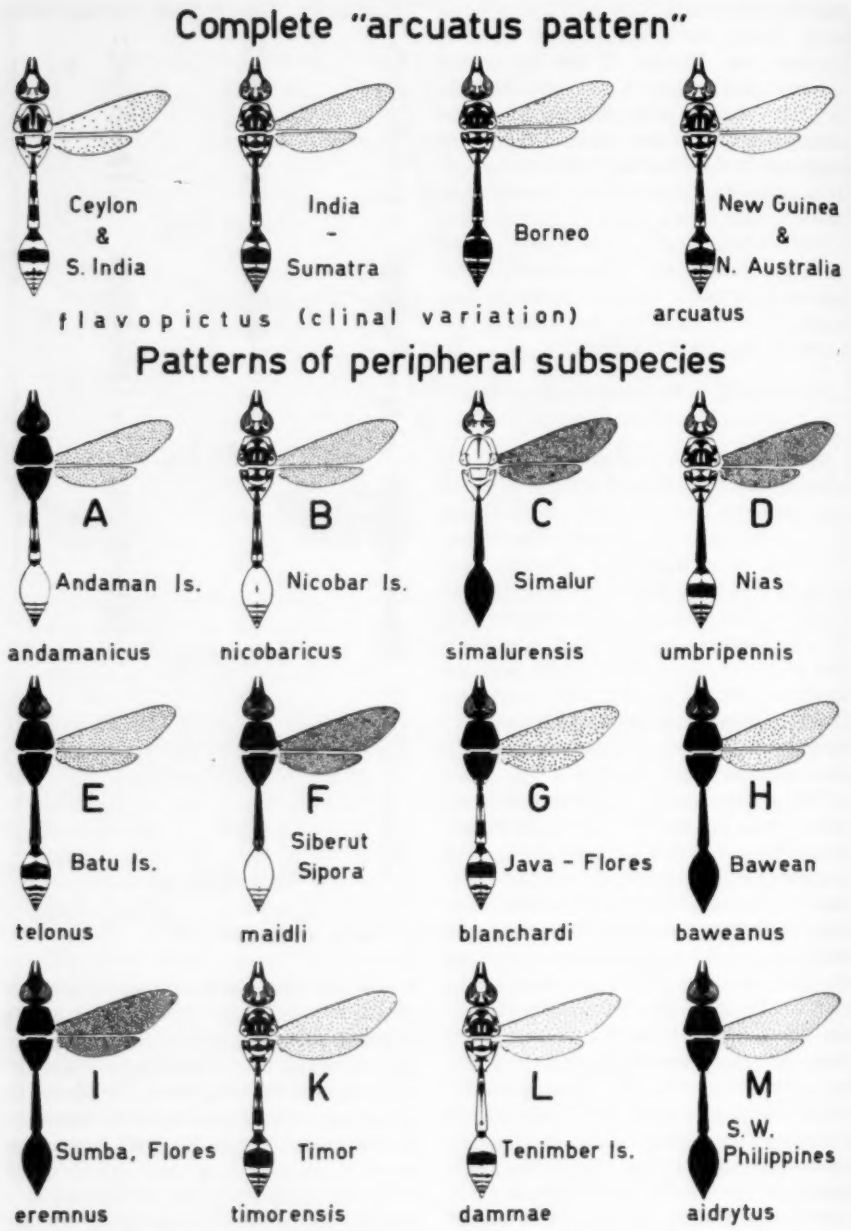


FIG. 4. Color patterns of *Eumenes*. Top row: mainland populations of *E. flavopictus* (showing clinal variation from west to east), and *E. arcuatus*, with similar color pattern; other rows: peripheral subspecies of *E. flavopictus* (compare fig. 5 and table 1).

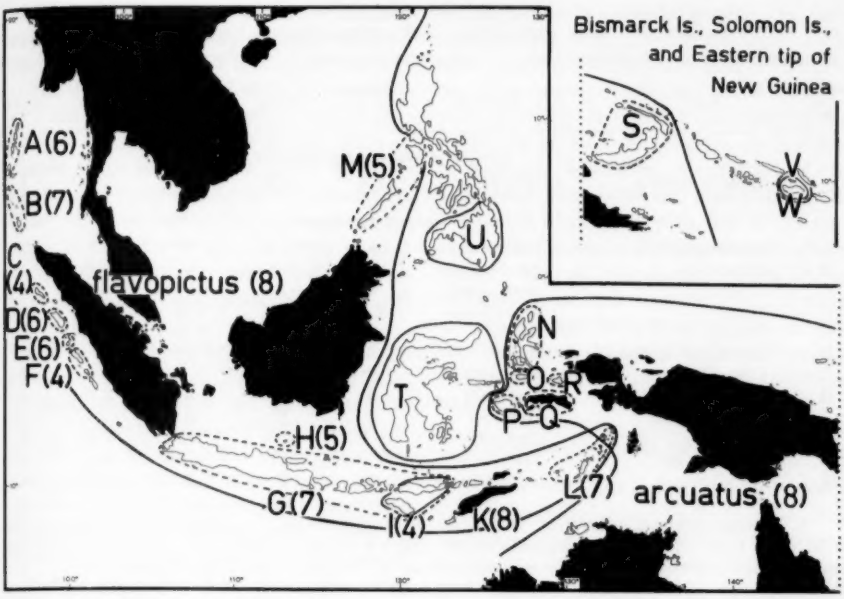


FIG. 5. Distribution of species of subgroup of *Eumenes arcuatus*. Black areas are inhabited by populations with complete black-and-yellow "arcuatus pattern" (top row of fig. 4). Numerals (in brackets) represent index of pattern (see table 1). Two poorly known species, related to T and inhabiting the Southern Moluccas (sympatric with P and Q), are omitted.

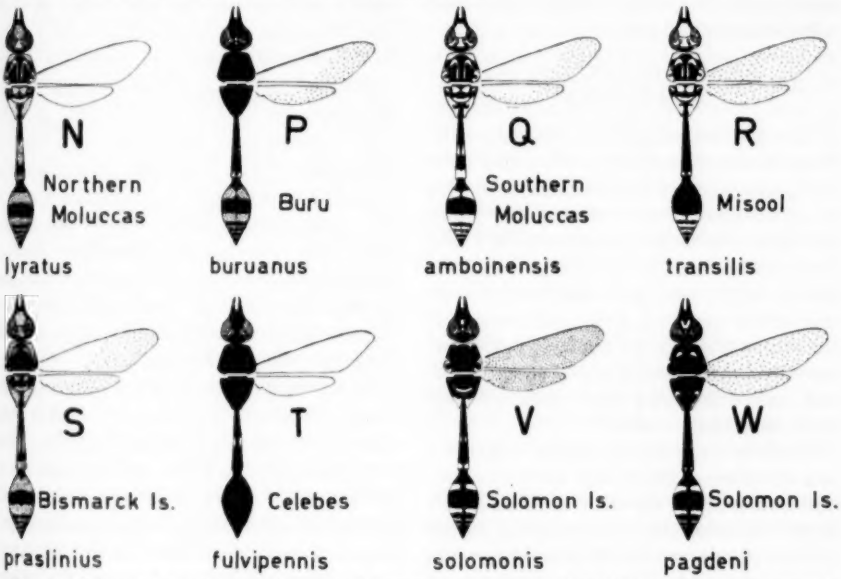


FIG. 6. Color patterns of five subspecies of *Eumenes arcuatus* (N–S) and of three related, monotypic, species.

TABLE 1. *Analysis of variation of color pattern in* Eumenes flavopictus *Blanchard* Y = yellow, B = black (dark wings), Y B = mixed pattern (fusco-hyaline wings)

On map (fig. 5):	Area	Subspecies	Head and thorax	Petiole	Gastral segm. 2–6	Wings	Index of pattern
		Central or mainland population					
	India to Borneo	*flavopictus*	Y B	Y B	Y B	Y B	8
		Peripheral populations					
A	Andaman Is.	*andamanicus*	B	Y B	Y	Y B	6
B	Nicobar Is.	*nicobaricus*	Y B	Y B	Y	Y B	7
C	Simalur I.	*simalurensis*	Y	B	B	B	4
D	Nias I.	*umbripennis*	Y B	B	Y B	B	6
E	Batu Is.	*telonus*	B	B	Y B	Y B	6
F	Sipora and Siberut Is.	*maidli*	B	B	Y	B	4
G	Java–Flores	*blanchardi*	B	Y B	Y B	Y B	7
H	Bawean I.	*baweanus*	B	B	B	Y B	5
I	Sumba, Flores	*eremnus*[1]	B	B	B	B	4
K	Timor	*timorensis*	Y B	Y B	Y B	Y B	8
L	Tenimber Is.	*dammae*	Y B	Y	Y B	Y B	7
M	S. Philippines	*aidrytus*	B	B	B	Y B	5

[1] *E. eremnus*, although structurally very similar to *E. flavopictus*, is considered to have reached specific rank; it is restricted to Sumba and Flores, where it occurs together with *blanchardi*, apparently without any interbreeding. This is evidently a case of "double colonization" (Mayr, 1942, p. 173), *blanchardi* being the more recent immigrant.

fauna of Timor is very incompletely known, and it seems likely that these less common members of the mimicry team will eventually be discovered there.

ANALYSIS OF VARIATION OF *E. FLAVOPICTUS*

In any given subspecies of *E. flavopictus* the pattern is remarkably stable. In a series of specimens it is always possible to find some variation in the extent of the yellow markings (for an example see van der Vecht, 1959, p. 35), but this intrasubspecific variation is only slight in comparison with the differences separating the subspecies. In particular it should be noted that aberrant individuals, comparable to any of the peripheral subspecies, have never been observed in the mainland population.

Analysis of the color patterns of the various subspecies (fig. 4 and table 1) shows that four parts of the body vary independently: (1) head and thorax, (2) the gastral petiole, (3) the remaining gastral segments, and (4) the wings. The first and third parts may differ from the pattern of the mainland form, the subsp. *flavopictus*, by being entirely black, or almost entirely yellow; the petiole is often entirely black, and in only one subspecies more extensively yellow than in the mainland form; the wings are either fusco-hyaline with a yellowish tinge, as in the mainland form, or dark brown. A very clearly yellow wing color is not found in the subspecies of *E. flavopictus*, although the brightly colored specimens from the western end of the area of distribution of the mainland form (Ceylon and Southern India) come close to it, but it is present in the closely related species from Celebes and the Papuan area, in combination with an all black, or yellow-and-black, body.

If we assign each of the populations an "index of pattern" (table 1) in such a way that in the four parts of the body the presence of yellow and black each is counted as one point, we arrive at the conclusion that this index is highest in the mainland population, and tends to fall off in the marginal populations, except in Timor. In other words, a uniformly balanced pattern is

maintained in the large central population, whereas in the periphery a loss in pattern has become established to various degrees.

The degree of loss is distinctly correlated with the degree of isolation, a relationship which is particularly clear in the chain of islands north and west of Sumatra. Concerning the latter it may be remarked that during the Pleistocene glaciations, when the ocean level was considerably lower than it is at present, Simalur and Siberut-Sipora were more peripheral than Nias and the Batu Islands.

It is evident that the degree of isolation is not only a matter of time, but that it also depends on the size of the population allowed by the area concerned. Thus the effects of a given period of isolation are more pronounced on smaller than on larger islands. Considerations of this kind may help to explain why the small island of Bawean has a relatively low "index of pattern," although this island may have become separated from the Sunda shelf more recently than certain other islands harboring populations with a higher index.

The presence of a subspecies with the complete *arcuatus* pattern in Timor is remarkable, particularly since Java and the western Lesser Sunda Islands are inhabited by a form with a reduced pattern. Any explanation of this difference is of course mere guesswork, since we know nothing definite about the size of the areas of distribution of these forms in the past. Yet the phenomenon seems less strange if we regard it against the background of the characteristic features of the faunas of these islands in general. The fauna of Timor is relatively rich as compared with that of some of the western Lesser Sunda Islands (Rensch, 1936; Mayr, 1944b), a fact which could point to a certain degree of stability necessary for the continued maintenance of the *arcuatus* pattern. On the other hand it is well known that Java's animal life is much poorer than one would suspect from its close proximity to Sumatra. Probably this is mainly due to pronounced volcanic activity during the Pleistocene (see Mayr, 1944a), which may have led to temporary, but very considerable, reductions of the inhabitable area. Such reductions may also have influenced the insect populations, which are often subspecifically different from those on the other side of the Sunda Straits. In the case of *Eumenes flavopictus*, the pattern of the subspecies *blanchardi* has undoubtedly developed in association with that of the corresponding forms of *Pareumenes* and *Pseumenes*. Any of these species separately, or perhaps two or three together, could at some time in the past have been reduced to very small numbers, thus passing through a stage likely to stimulate reduction of the color pattern.

Possible Genetical Interpretation

Mayr (1954) has shown that peripherally isolated populations are likely to differ from the parent population by depleted genetic variability. His views were confirmed by Dobzhansky (1957), who demonstrated that in the South American *Drosophila willistoni* the peripheral populations are indeed characterized by genetical losses, in this case consisting of a marked reduction in chromosomal inversions, the reduction being most pronounced in the smaller islands.

On this basis, it may be assumed that the observed losses in the "index of pattern" in the peripheral populations of *E. flavopictus* can likewise be regarded as an expression of genetical depauperization. If this were true, we would have here a striking example of this phenomenon, for only rarely will it be possible to read the effects of isolation so clearly from the characters of the phenotypes.

Unfortunately, even if it were possible to breed these wasps in captivity, an experimental genetical analysis is not feasible at present. Any attempt at a genetical interpretation must therefore remain purely hypothetical. Nevertheless, some support might be found in the outcome of investigations on the genetics of color patterns in other insects. Tan (1946) found that the distinctive types of the elytral pattern in the ladybeetle *Harmonia axyridis* are dependent

on a multiple-allelic series of genes, and da Cunha (1949) showed that in *Drosophila polymorpha* the three concurrently existing color types of the abdomen, dark, light, and an intermediate, are caused by a single pair of alleles (EE, ee, and Ee respectively).

Since in *Eumenes flavopictus* the three types of coloration (Y, B, and YB, in table 1) are quite distinct and by no means arbitrarily chosen from an intergrading series, a genetic model might suggest itself in the assumption that the intermediate type YB is the heterozygote, and Y and B are the two homozygous types of a single pair of allelic factors.

However, such a scheme seems unlikely on the following grounds. First, heterozygosity where present would have to be maintained by a highly uneconomical or involved mechanism for eliminating or preventing homozygous combinations. Secondly, it would require the assumption of four pairs of alleles, all similar, but each restricted to a particular part of the body.

In accordance with the interpretation proposed by Mayr (1954), one might expect more reasonably that the color characters depend on the interaction of the total genetic background with a limited number of genes determining the pigmentation, and that the background genes are those primarily affected by the gene loss resulting from the isolation of small parts of the population.

As we have seen, there are good reasons to accept that allaesthetic selection in the mainland subspecies has a stabilizing effect on the color pattern. The balance between opposing tendencies to pigmentation is maintained in these large populations, even against the undoubtedly different genetic backgrounds of the two species, *E. flavopictus* and *E. arcuatus*.

When small parts of such a balanced population become isolated, there will occur changes in the genetic system due to random drift, but at the same time the isolated units will be subjected to selection pressures likely to differ somehow from those operating on the mainland. The originally existing balance may thus be disturbed in two ways, and in both ways the deviations from the original condition are likely to be greater in small population units. If such a unit survives, its genetic system will evolve in the direction of a new equilibrium through the interaction between genetic drift and natural selection (Dobzhansky and Pavlovsky, 1957). Evidently this is how the peripheral subspecies of *E. flavopictus* have developed, for they present a striking example of the divergence to be expected from the results of such interaction in different small populations. Mayr and Vaurie (1948) regard the unpredictability of the variation as one of the characteristics of isolated peripheral populations. In these wasps the independent changes of the color pattern in various parts of the body are particularly remarkable; they suggest that the deposition of pigment in different parts of the body is at least partly governed by different genes.

Concluding Remarks

Up to the present no *Pareumenes* and *Pseumenes* have been found in the chain of islands north and west of Sumatra. This suggests that in these islands the various *Eumenes* patterns have become established independently. However, the wasp fauna of this area is still incompletely known. If *Pareumenes* or *Pseumenes*, which are always rarer than *Eumenes*, are eventually found in these localities, we may expect their color patterns to agree with that of the subspecies of *Eumenes* occurring in the same island or island group.

The variation of the peripheral subspecies of *Eumenes arcuatus* (fig. 6) is rather different from that of *E. flavopictus*. In three subspecies inhabiting the Northern Moluccas and the Bismarck Archipelago the yellow color is replaced by orange brown, in the Misool subspecies the first band of the second gastral tergite is reduced or absent, and only the Buru subspecies has a pattern which is very similar to that of one of the *flavopictus* subspecies (subsp. *blanchardi*). The generally deviating evolutionary trends of the isolates of *E. arcuatus* seem to suggest that this species is

genetically less similar to *E. flavopictus* than would appear from the color pattern of its central population, thus confirming the conclusions based on morphological characters.

Summary

The Indo-Australian species of the group of *Eumenes arcuatus* may be regarded as the product of two successive invasions of Asiatic origin. The first invasion is mainly represented by rare forest inhabitants, the second by two widely distributed, common and polytypic species (*E. flavopictus*, oriental, and *E. arcuatus*, Australian) and six local, insular and monotypic species. The two polytypic species both consist of a central population inhabiting an extensive area, and a number of more or less differing peripheral subspecies. The striking similarity in color pattern of the two central populations is ascribed to the stabilizing effect of allaesthetic selection favoring a pattern with highly efficient warning capacity; the deviating patterns of the peripheral subspecies of *E. flavopictus* are considered to result from depleted genetic variability caused by different degrees of isolation.

Acknowledgments

The author is deeply indebted to Professor Ernst Mayr for various valuable suggestions, and to Professor H. J. Gloor and Mr. W. Scharloo, Genetical Institute, Leiden University, for help in discussing the genetical interpretation of the data.

Literature Cited

Cott, H. B. 1954. Allaesthetic selection and its evolutionary aspects. *In*: Huxley, Hardy & Ford, Evolution as a process: 47–70.

da Cunha, A. B. 1949. Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. Evolution, **3**: 239–251.

Darlington, P. J., Jr. 1957. Zoogeography: the geographical distribution of animals. Wiley, New York.

Dobzhansky, T. 1957. Genetics of natural populations. XXVI. Chromosomal variability in island and continental populations of *Drosophila willistoni* from Central America and the West Indies. Evolution, **11**: 280–293.

—— and Olga Pavlovsky. 1957. An experimental study of interaction between genetic drift and natural selection. Evolution, **11**: 311–319.

Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.

——. 1944a. Wallace's line in the light of recent zoogeographic studies. Quart. Rev. Biol., **19**: 1–14.

——. 1944b. The birds of Timor and Sumba. Bull. Amer. Mus. Nat. Hist., **83**: 123–194.

——. 1954. Change of environment and evolution. *In*: Huxley, Hardy & Ford, Evolution as a process: 157–180.

—— and Charles Vaurie. 1948. Evolution in the family Dicruridae (Birds). Evolution, **2**: 238–265.

Rensch, B. 1936. Die Geschichte des Sundabogens. Gebr. Borntraeger, Berlin.

Ruiter, L. de. 1959. Some remarks on problems of the ecology and evolution of mimicry. Arch. Néerl. Zool., **13**, Suppl.: 351–368.

Tan, C. C. 1946. Mosaic dominance in the inheritance of color patterns in the ladybird beetle, *Harmonia axyridis*. Genetics, **31**: 195–210.

Vecht, J. van der. 1937. Descriptions and records of Oriental and Papuan solitary Vespidae. Treubia, **16**: 261–293.

——. 1959. On *Eumenes arcuatus* (Fabricius) and some allied Indo-Australian wasps (Hymenoptera, Vespidae). Zool. Verh. Rijksmus. Nat. Hist. Leiden, **41**: 1–71.

Zimmermann, K. 1931. Studien über individuelle und geographische Variabilität paläarktischer *Polistes* und verwandter Vespiden. Zeitschr. Morph. Oekol. Tiere, **22**: 173–230.

FOOD-PLANT SPECIALIZATION IN THE MOTH *PANAXIA DOMINULA* L.

L. M. Cook
Department of Zoology, University of Oxford[1]

Received March 6, 1961

The larvae of *Panaxia dominula* have been recorded on many species of plants in the field. There is evidence that preference for comfrey (*Symphytum officinale* L.) occurs in Britain, although some colonies of the moth are known in which it is not present. In central Europe the larvae are seldom or never found on comfrey, so this may be an example of food-plant specialization within a section of an otherwise polyphagous species. Such specialization, while restricting the variety of habitat available, may increase the overall fitness of the populations in which it occurs. As part of an investigation into the ecology of the insect a study of its feeding habits has been made. Some observations on larval behavior and food preferences are discussed here.

Data

Food plants.—Plants on which *dominula* has been observed to feed are listed in table 1. Most of the records are from the field, and they are tabulated according to family and the region from which they come. In Britain over 20 species belonging to nine families have been noted, but the most generally favored in wild populations appears to be comfrey. Of 17 extant colonies which I have examined in England and Wales, 12 were established around a supply of this plant. Comfrey usually grows in small clumps, and when there is a colony of *dominula* on it, they are found at high densities on the plants in the clump although many also feed on the surrounding vegetation.

Where comfrey is absent the larvae do not show a preference for a single alternative species. At Axmouth, Devon, they feed equally on bramble (*Rubus* spp.) and on stinging nettle (*Urtica dioica*), while at Kingsdown, Kent, they may be seen in early spring feeding in large numbers on burdock (*Arctium* sp.). In this colony *Arctium* is eaten in April while the leaves are still small, but as the year proceeds the larvae move off it and are found mainly on nettle. This is probably because the leaves of *Arctium* become too tough as they get large; even when they are small the insects only graze each side and do not perforate them, as is the usual practice. At Llancynfilyn, Cardiganshire, the principal larval food-plant is dwalf sallow; while at Milton Combe, Devon, there is a small colony which feeds on evergreen alkanet (*Pentaglottis sempervirens*).

Thus, although comfrey is frequently present, it does not appear to be essential. Where the larvae feed on it many individuals are found on other plants, and it will be shown below that in a colony where the food of choice appears to be comfrey, its contribution to the total bulk consumed may be in fact quite small.

The colony at Cothill, Berks.—The Cothill colony is situated in a small region of fen and carr surrounded by agricultural land (for a description see Fisher and Ford, 1947). The larvae are most easily found by searching the comfrey plants which grow in a number of distinct aggregates. A series of quadrat samples of both comfrey and larvae have been taken to determine their exact distribution within the colony and their number and density in different parts of it. Some of the results, which indicate the degree to which the position of comfrey has influenced the distribution of larvae, are presented here.

An estimate of the number of comfrey plants present and their position was obtained by counting the number in belt transects running N–S across the area. The data are presented graphically in fig. 1. The sampling fraction was 1 in 4.375, so the pic-

[1] Present address: Department of Zoology, University of Leicester, Leicester, England.

TABLE 1. *Food-plants of* dominula *larvae*

Family	Britain	France	Rest of Europe
Ranunculaceae			*Ranunculus* sp.
Geraniaceae			*Geranium* sp.
Rhamnaceae			*Frangulus ulnus* Mill.
		Genista sp.	
Rosaceae	*Crataegus* sp.		*Fragaria vesca* L.
	Prunus spinosa L.		*Prunus padus* L.
	Rosa sp.		*Rubus idaeus* L.
	Rubus spp.		*Rubus* spp.
	Filipendula ulmaria (L.)		*Spiraea salicifolia* L.
	Fragaria vesca L.		
Urticaceae	*Parietaria diffusa* Mert. & Koch	*Urtica* sp.	*Urtica* sp.
	Urtica dioica L.		
Cannabinaceae	*Humulus lupulus* L.		
Ulmaceae			*Ulmus* sp.
Betulaceae			*Alnus* sp.
Salicaceae	*Salix caprea* L.		*Salix* sp.
			Populus sp.
Oleaceae	*Fraxinus excelsior* L.		*Fraxinus* sp.
Boraginaceae	*Cynoglossum officinale* L.		*Myosotis palustris* L.
	Symphytum officinale L.		*Cynoglossum* sp.
		Symphytum sp.	
	Pentaglottis sempervirens (L.)		
Labiatae	*Lamium album* L.		*Lamium* sp.
	Mentha aquatica L.		
Plantaginaceae	*Plantago* sp.		
Caprifoliaceae			*Lonicera* sp.
Compositae	*Arctium* sp.		
	Eupatorium cannabinum L.		
	Senecio vulgaris L.		
	various thistles		

Sources: Kettlewell (1942), Lhomme (1923), Schneider (1926), Seppänen (1954), personal observation and correspondence. The nomenclature used is that of Clapham, Tutin, and Warburg (1952).

ture which emerges from the estimation probably represents accurately the actual distribution. In winter the larvae hibernate in dry litter, including dead comfrey leaves, and during this period they constitute a static population which may be sampled by means of quadrats. In the winter of 1957–58 the hibernating population was examined using quadrats of 3-foot side on a 70-foot grid (fig. 2). Although the sampling fraction is in this case very small, the pattern indicated is probably a fair approximation to the true distribution of larvae. Part of the area was sampled at 35-foot intervals, and the 35-foot and the 70-foot grid both indicate the same distribution. The frequency distribution of both plant and insect differ significantly from the Poisson distribution for the observed means, indicating clumping of individuals. Figs. 1 and 2 represent the situation with respect to the same two pairs of coordinates, so that the distribution of plant and insect may be compared. It is evident that the clumps do not always coincide; 26 of 34 comfrey plants falling within transects in sectors A1 and A2 occur in a dense aggregate in A1 on the edge of an extensive bed of the reed *Phragmites communis*, while 52 of 65 larvae sampled in the same sectors were found in the reed bed beyond the comfrey. The latter represents 48% of the total number of larvae found in the quadrats. In one other area a high density of the insects corresponds more closely with a patch of comfrey, and it is possible that larvae found in the reeds had moved

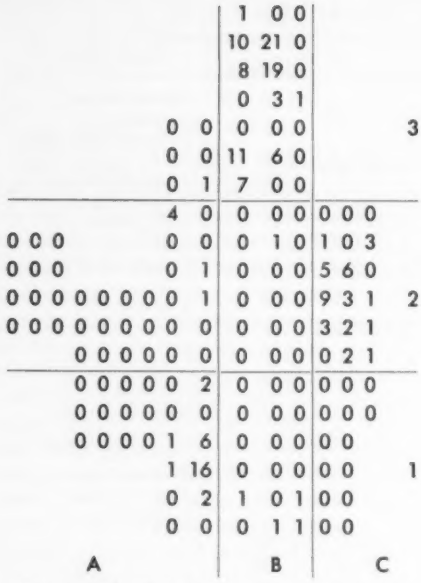


FIG. 1. Distribution of comfrey at Cothill. Numbers indicate numbers of plants found in belt transects on the sampling grid.

FIG. 2. Larval hibernation, 1957–58. Numbers indicate numbers of insects found in quadrats situated on the sampling grid.

there to hibernate after feeding on comfrey. If this were so, samples of larvae collected in the reeds at the onset of hibernation would be expected to weigh approximately the same as those taken from the comfrey at the same date. The weights would also be similar if the larvae in the two areas did not migrate from one to the other, (a) if the position and types of food have no effect on weight, or (b) if they do affect weight but hibernation commences only when a particular stage has been attained. If the weights of the two samples differed, however, it would suggest that they were drawn from separate groups which fared differently in their respective localities. Two samples of 20 larvae collected on 12.11.58 weighed respectively 24.98 ± 9.17 mg on comfrey, and 16.03 ± 6.34 mg in the reeds. The difference is significant ($t = 3.59$, $P < 0.05$). Samples taken after hibernation is completed and feeding has recommenced show that the difference in weight, which also reflects a difference in the stage of development, is maintained.

It seems probable, therefore, that larvae collected in the reeds remain there throughout development and never eat comfrey. They feed mainly on *Filipendula ulmaria*, and also on *Eupatorium cannabinum*. The data obtained by sampling suggest that this applies to a fraction approaching half of the

Date	On comfrey		In reeds		t	P
	Weight	No.	Weight	No.		
21.4.59	97.67 ± 44.53	12	47.36 ± 12.24	7	3.68	< 0.01
21.5.59	295.90 ± 104.68	15	130.39 ± 56.30	14	5.35	< 0.001
5.11.58	28.68 ± 6.33	20	18.36 ± 6.00	38	6.02	< 0.001
4.6.58	313.14 ± 69.25	38	246.15 ± 90.00	26	3.20	< 0.01

total population at Cothill. Estimation of the numbers on the comfrey plants themselves was not carried out on this occasion, but it was on three other occasions when the grid sample was repeated, giving values between 7% and 12% of the estimates derived from the grid. Assuming the same to hold on this occasion, the data indicate that at least 40% and perhaps as many as twice that fraction of the larvae do not feed on comfrey although the colony is apparently based on it.

The success of colonies where comfrey is absent.—If comfrey is not essential for the establishment of a colony it may be asked what advantages there are associated with its presence. The size of a colony appears to bear no close relation to the presence or absence of comfrey. The colony at Kingsdown, Kent, is well known for the large numbers to be seen flying there (Kettlewell, 1942). In July 1959 moths were very abundant at Axmouth, at another colony where there is no comfrey. I made an estimate of the population size for one day by the capture-recapture method which gave a mean value of about 4,500. Since all the marked recaptures were caught when the releasing area was searched, indicating inadequate dispersal, estimation was not continued, but for the same reason the value obtained is probably a minimum one and the great numbers seen suggest a much higher figure. By comparison the seasonal population size at Cothill since 1941 has frequently been as small as or smaller than 5,000 (Fisher and Ford, 1947; Sheppard, 1951, 1956).

As indicated above, the larvae which feed on comfrey at Cothill grow more rapidly than those which do not, but this difference could conceivably be due as much to the presence of reeds, shading the food-plants from the sun, as to any relative dietary deficiency of other plant species when compared with comfrey. A small series of observations on captive larvae fed from the penultimate instar exclusively either on comfrey or on stinging nettle has failed to reveal differential survival on the different food-plants even though total survival was low (Cook, 1961).

Larval preference.—So far, no direct evidence has been obtained to support the belief that the species is more successful when comfrey is present than when it is absent, and the frequent association between the plant and colonies of *dominula* might be put down to restriction of both to the same type of habitat. This is very probably the explanation of the association commonly found between *dominula* and *Phragmites communis*, but since the eggs may be distributed across the vegetation without particular reference to any single plant species (Sheppard, 1951), the presence of larvae in large numbers on *Symphytum* must be due to their ability to discriminate between plants. It is of interest, therefore, to know whether any difference exists between those from "comfrey" and from "non-comfrey" colonies in their reactions towards comfrey.

As a preliminary test, larvae from Kingsdown, where there is no comfrey, were kept in a large container and given an abundant supply of both comfrey and stinging nettle, the plant on which they are most frequently found. The number on each plant was recorded on 27 successive occasions. The position of the two plants was reversed three times so as to minimize any possible effect of situation on choice. More individuals were observed on comfrey than on nettle, and although there is great variation between successive observations, the excess on comfrey is significant ($P < 0.01$). Details are given in table 2. Since the evidence obtained in this way suggests that they do exercise a preference, and furthermore that it may be for a plant on which they do not normally feed, a comparison was made between larvae from a comfrey and a non-comfrey colony.

The colonies chosen were Cothill, where the larvae feed on *Symphytum officinale* and *Filipendula ulmaria*, and Axmouth, where the usual foods are *Urtica dioica* and *Rubus*. The plants presented to them were *Symphytum officinale*, *S. caucasicum*,

TABLE 2. *Number of larvae observed on nettle and comfrey when both were available*

	Position 1			Position 2	
	Comfrey	Nettle		Comfrey	Nettle
a.	36	5	a.	4	9
	18	27		13	27
	12	20		11	13
	29	12		27	21
	20	10		28	15
	22	10		26	7
				14	22
Total	137	84		13	22
b.	14	5	Total	136	136
	26	8			
	23	4	b.	26	4
				18	15
Total	63	17		10	1
				8	4
				8	4
				4	3
				4	4
Total $\chi^2_{(27)} = 111.83$,				5	2
$P < 0.001$				9	0
$\chi^2_{(1)}$ of total $= 34.52$,				3	0
$P < 0.001$					
			Total	95	37
			Sum total	431	274

TABLE 3. *Analysis of variance for loss in weight by evaporation in different plant species*

	d.f.	S.s.	M.s.	V.r.	*P*
Between groups	4	710.49	177.62	1.06	>0.20
Within groups	35	5888.56	168.25		

Urtica dioica, *Filipendula*, and *Rubus*. *S. caucasicum* Bieb. was selected because it is very similar to *S. officinale*. It is not yet naturalized here but like *S.* × *uplandicum* Nyman, which is now a member of the British flora, it differs slightly in habitat from *S. officinale*. If these species are acceptable to the larvae their introduction in Britain may have affected the distribution of the insect here.

Tests of food preference were carried out by presenting samples of five larvae with weighed whole leaves of *S. officinale* and of the alternative food. Approximately equal quantities of each plant species were weighed, given to the larvae, and the uneaten portions reweighed about 20 hours later. The weights eaten were obtained by subtraction, and in table 4 they are expressed as the percentage of the total food eaten which was not *S. officinale*. In order to reduce the effects of individual preferences the larvae were redistributed after each trial so that they received a different alternative plant. Eight trials were carried out for each comparison within each colony.

A further possible source of error arises from the fact that the leaves of different plants may lose moisture at different rates. Since this loss is included in the weight attributed to larval feeding, it would affect the results if the rates were appreciably different. To investigate this possibility a pair of leaves was weighed along with each series of trials. Analysis of variance shows that within the terms of this experiment the rates of loss in weight by the leaves of the different plant species did not differ significantly (table 3).

The results of the experiment are given in table 4. Although there are distinct differences in the acceptability of the plants offered, and in their attractiveness to larvae from the different colonies, there is in both colonies a preference for *S. officinale*. In all cases the weight eaten of the alternative food is less than 50% and in four instances it is significantly less. There is no significant difference in this respect between the larvae from the two colonies. A greater weight of *S. officinale* than of any other single plant species was offered, so it is possible that the larvae develop a preference for the most abundant food. This would not account for the result of the previous experiment, however, and there was no evidence that the relative weight of comfrey eaten increased during the course of the experiment.

THE SITUATION IN OTHER PARTS OF THE RANGE

Lhomme (1923) records the larvae of *dominula* on *Symphytum* in France, and M. Bourgogne informs me that it is well known there to be a food-plant of the species. This does not appear to be so, however, in other

TABLE 4. *Weight of different plants eaten when* S. officinale *was also present, expressed as the percentage of the total food eaten. The value of* P *gives the likelihood of an observation exceeding 50*

	S. caucasicum	*Urtica*	*Filipendula*	*Rubus*
Axmouth	30.94 ± 15.42	23.04 ± 14.96	13.49 ± 8.30	23.33 ± 17.81
P	> 0.05	0.05 – 0.01	< 0.001	> 0.05
Cothill	32.54 ± 16.42	38.76 ± 18.92	8.52 ± 6.47	29.33 ± 7.98
P	> 0.05	> 0.05	< 0.001	0.01 – 0.001
t (between colonies)	0.201	1.910	1.337	0.871
P	>0.50	> 0.05	> 0.10	> 0.10

parts of the range. In Spain, where the moth is widespread in mountain regions, it has not been recorded from *S. officinale* although that plant is common. In Italy *Symphytum* is not regarded as a typical larval food-plant, neither is it in Czechoslovakia, where the larvae are found most frequently on *Urtica dioica* and *Salix caprea*. These two species appear to be typical food-plants in central Europe, although many others are eaten. Schneider (1926), who discusses the food-plants of *dominula* at some length, does not mention *Symphytum*. It is not recorded from Sweden, Denmark, or Germany in Seppänen's (1954) list, which draws on authoritative earlier sources. Unless comfrey is much less common in other parts of Europe than it is in Britain, which is probably not the case, it may be concluded that the feeding habits of *dominula* are not the same in all parts of the geographical range.

DISCUSSION AND CONCLUSIONS

Over most of the range the larvae appear to be polyphagous, with a preference, if any is expressed, for *Urtica dioica* and *Salix* spp. In France and Britain, however, they are frequently found on *Symphytum*, and experimental evidence indicates that, when a choice is available, a preference for *Symphytum* is exhibited even by individuals from colonies established in places where it is not present. A genetic basis for the preference is probable, therefore, in all populations in this region, and not only where it is manifest.

Although it is frequently advantageous for a species to be unspecialized so that it may utilize the resources of a wide range of habitats, there are circumstances in which this advantage is outweighed by the benefits of specialization. Brower (1958) suggests that some related sympatric insect species feed on different food-plants because the behavior of the predators would lead to greater loss if they shared common food-plants. The predators are agents initiating the establishment of divergent selective pressures in the two species. Divergent selection due to pressure of competition has been discussed many times in birds (Lack, 1954; MacArthur, 1958; Vaurie, 1951), and Lack (*ibid.*) has argued that the tendency for related sympatric species of birds to eat different food shows that their numbers are limited by food supply.

Habitat restriction can also take place when other aspects of the environment become unfavorable. Changing environmental conditions which lead to decrease in the size of a population cause reduction in the potential genetic variability and restriction to a limited optimum habitat. The edge of a species range represents an extension in space, as well as time, of such conditions. Food-plant specialization on the edge of the range may be a means of keeping an insect within an optimum habitat, although the food itself is not necessarily the optimum component. This is perhaps the purpose of specialization on *Peucedanum palustre* by *Papilio machaon* in Britain (see Ford, 1953). In captivity the larvae will eat other species of Umbelliferae. Wild larvae are almost always found on *P. palustre* because the female selects this plant for oviposition. As a result of this preference, and of an accom-

panying reduction in the tendency of the adults to wander, the life cycle takes place within a restricted habitat, of which *P. palustre* happens to be typical. Selection for food-plant specialization could take place if conditions elsewhere were unfavorable for reasons which need have no connection with the quality of this particular plant as food.

In *dominula* the female does not carefully choose a plant on which to oviposit, but scatters the eggs freely over the breeding area; the preference is a larval, not an adult one. If colonies are slightly more successful when established on *Symphytum* than they are on other plants, then conditions encountered in marginal habitats will tend to restrict them to *Symphytum*. Preference for this plant on the part of the larvae would be favored whether the advantage accrued to the larvae themselves or to some other stage in the life of the individuals. Limitation of numbers is associated with food supply, and if the species can maintain itself successfully on comfrey in a marginal habitat, where it cannot do so on other plants, then the nature of the food-plant is the decisive factor allowing colonization.

In Britain, *dominula* is a strictly colonial insect, the colonies falling into two broad types of habitat. They are, (a) agriculturally marginal land, usually coppiced, with high atmospheric humidity and fairly damp ground, and (b) hedgerows. The important ecological factors which contribute to success in each facies are unknown, but the hedgerow colonies are both fewer in number and smaller in size than the others. They possess none of the features associated with the first except that they are always established on comfrey. It provides nearly all the food and larval hibernacula in them, whereas in the first type it may be absent, or, as at Cothill, may play a comparatively small role. It is perhaps worth mentioning here one observation which has a possible bearing on its selection as a food-plant. Comfrey is a hemicryptophyte (Raunkiaer, 1937) which has a thick covering of withered remains lying on top of the overwintering bud. The expansion of the new shoot into this dead material begins very early in winter, with the result that by the end of February there are about two inches of new vegetation above ground, while the new growth on most other plants has not begun to appear. At the beginning of March, when the usual alternative food-plants are still very small, the larvae become active and attack the growing shoots. At Cothill in the spring of 1958, when larval density was, for that colony, very high, up to three dozen larvae were to be found around individual shoots which were eaten down to the ground. As a result of this activity the plants within the colony were about five inches high by the middle of May, when comfrey plants nearby which had had no larvae on them were about thirteen inches high. During the first half of March there must have been some mortality due to food shortage; but it would have been considerably greater had comfrey been absent. Under climatic conditions in which larval hibernation terminates before regrowth of vegetation is well under way, the presence of the early growing shoot may be one of its important attributes.

Summary

1. The larvae of *P. dominula* eat many species of plants, but in Britain the majority of colonies appear to be established on comfrey (*Symphytum officinale*). When they are not, no single alternative plant is favored.

2. Evidence is given for one colony (at Cothill, Berks.), where the food of choice appears to be comfrey, showing that at least 40% of the larvae never feed on it. Colonies in sites where there is no comfrey are often as large and successful as those where it is present.

3. Experiments show that the larvae exhibit a preference for comfrey even when they come from colonies where it is absent.

4. Britain and France are the only parts of the range where comfrey is considered to be a food-plant of this insect. The significance of the apparent specialization in a region on the western edge of the range is

discussed, and a possible advantage is suggested.

ACKNOWLEDGMENTS

I am deeply indebted to all those who have provided information from their observations on the biology of the moth. In particular I would like to thank Mr. F. W. Jeffery and Mr. E. C. Pelham-Clinton for their help with regard to colonies in England and Wales, and, for their comments on the situation in other parts of Europe, the following: Dr. R. Agenjo, M. J. Bourgogne, Herrn. F. Daniel, Professor K. Hrubý, Dr. J. Klimesch, Professor M. Mariani, Dr. D. Povolný, Dr. S. Toll, and Dr. S. Zangheri.

My thanks are due to Dr. A. J. Cain for his help and comments during the course of this work, and to Dr. E. B. Ford and Dr. H. B. D. Kettlewell who criticized the paper in manuscript. The work was carried out during the tenure of a grant from the Nature Conservancy.

LITERATURE CITED

BROWER, L. P. 1958. Bird predation and food-plant specificity in closely related procryptic insects. Amer. Nat., **92**: 183–187.

CLAPHAM, A. R., T. G. TUTIN, AND E. F. WARBURG. 1952. Flora of the British Isles. Cambridge University Press.

COOK, L. M. 1961. Differential mortality between larvae of *Panaxia dominula* from different colonies. Entomologist, **94**: 135–136.

FISHER, R. A., AND E. B. FORD. 1947. The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. Heredity, **1**: 143–174.

FORD, E. B. 1953. Evolution in polymorphic forms. Proc. 9th Int. Congr. Genet., 463–468.

KETTLEWELL, H. B. D. 1942. A survey of the insect *Panaxia* (*Callimorpha*) *dominula* L. Proc. S. Lond. Ent. Nat. Hist. Soc. **1942–43**: 1–49.

LACK, D. 1954. The natural regulation of animal numbers. Oxford, Clarendon Press.

LHOMME, L. 1923. Catalogue des Lépidoptères de France et de Belgique. Lhomme. Le Carriol, Par Douelle.

MACARTHUR, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. Ecology, **39**: 599–619.

Raunkiaer, C. 1937. Plant life forms. Oxford, Clarendon Press.

SCHNEIDER, H. 1926. Von der *dominula*-Raupe. Int. Ent. Z. Guben., **20**: 298–300, 315–316.

SEPPÄNEN, E. J. 1954. Suomen Suurperhostoukien Ravintokasvit. Porvoo, Helsinki.

SHEPPARD, P. M. 1951. A quantitative study of two populations of the moth *Panaxia dominula* (L.). Heredity, **5**: 349–378.

——. 1956. Ecology and its bearing on population genetics. Proc. Roy. Soc. Lond., (B) **145**: 308–315.

VAURIE, C. 1951. Adaptive differences between two sympatric species of Nuthatches (*Sitta*). Proc. X Int. Orn. Congr., **1950**: 163–166.

PECULIAR GENE ARRANGEMENTS IN BRAZILIAN NATURAL POPULATIONS OF *DROSOPHILA ANANASSAE*[1]

N. FREIRE-MAIA
University of Paraná, Curitiba, Brazil

Received September 19, 1960

Paracentric inversions are theoretically known as disadvantageous events in species which have crossing over in both sexes. In Diptera, however, the absence of crossing over in males and the selective elimination of dicentric and acentric chromatids during meiosis in females, created the possibility for the group to use this type of chromosome mutation in the structuring of their populations. Paracentric inversions are, therefore, the most widespread mechanism for change of gene sequence in *Drosophila*. Other mechanisms, such as pericentric inversions and translocations, although known to have played an important role in the phylogeny of a large number of species in the past, seem to be operating very infrequently on the natural populations of present day species, and so present a contrasting picture with paracentric inversions.

The present paper will describe the peculiar situation of *Drosophila ananassae*, in which it has been possible to detect a series of chromosome mutations practically non-existent in a number of other species belonging to the same genus. The data on paracentric inversions (summarized in Freire-Maia, 1955) will be presented in a separate paper.

MATERIAL AND METHODS

Drosophila ananassae Doleschall, a member of the *melanogaster* group, is a cosmopolitan species adapted to domestic habitats. In Brazil it is easily found, sometimes with frequencies as high and higher than 50%, in localities characterized by hot climates, such as those on the littoral and in central and northern Brazil. In inland southern localities, however, known for very cold winters and mild summers, the species has not been found or was extremely rare in several collections made over about five years. Other species belonging to the domestic Brazilian fauna are *D. simulans*, *D. melanogaster*, *D. immigrans*, *D. kikkawai*, *D. busckii*, *D. hydei*, *D. repleta*, and *D. prarepleta*. *D. ananassae* is, however, among them, the species possessing the highest degree of chromosome polymorphism (Freire-Maia, 1955).

All flies were collected in domestic habitats such as markets and orchards, and captured generally on fruits normally occurring in such places.

Preparations by the usual orcein method have been used. The vials with flies were kept at approximately 25° C. Quantitative data were obtained through the analysis of only one larva from each female captured in nature. Non-fertilized or sterile females were discarded.

DATA

The Salivary Gland Chromosomes

The salivary gland nuclei of *D. ananassae* present six euchromatic arms extending from the chromocenter, four being long (corresponding to the metacentric II and III chromosomes), and two short corresponding to both arms of X chromosome (cf. Kikkawa, 1938; see the chromosome map by Seecof, 1957). The metacentric IV chromosome, being almost entirely heterochromatic, is embedded in the chromocenter and does not present any easily observable euchromatic constituent.

D. ananassae is very favorable material for cytogenetical studies. It breeds easily on the usual banana–agar medium and its

[1] This investigation was supported in part by grants from the National Research Council of Brazil and from the Rockefeller Foundation.

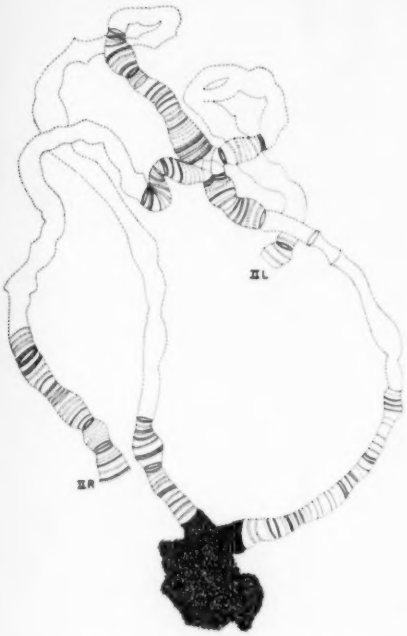


FIG. 1. Pericentric inversion *a* (chromosome II).

FIG. 2. Pericentric inversion *a* (chromosome III).

salivary gland chromosomes are extremely suitable for analysis.

The nomenclature proposed by Kikkawa (1938) for the salivary gland chromosomes will be used in this paper.

Gene Arrangements

Through the analysis of 1,377 larvae, each one the offspring of one female fertilized in nature, a total of 19 different paracentric inversions, 5 pericentric inversions, 1 translocation, 2 transpositions (shifts), 1 deletion, and several duplication-like terminal "extra bands" have been found. This list confers on *D. ananassae* a very peculiar place within the genus. A study of the paracentric inversions as well as of the "extra bands" will be made elsewhere. This paper will describe and discuss only problems referring to the other types of gene arrangements.

Pericentric Inversions

The five pericentrics found are represented in figs. 1–5, and may be roughly described as follows:[2]

Inversion a (II chromosome). — Very long, it is the only pericentric found on this chromosome (fig. 1). Slightly asymmetrical, it includes about half or less of the right arm and about ¾ or less of the left arm. It was found together with the so-called IIIL "terminal" inversion first described by Kaufmann (1936a).

Inversion a (III chromosome). — Also

[2] The inversions named A, B, C, D, and E in the preliminary notes published on this problem (cf. Freire-Maia, 1953, 1954) correspond respectively to those here called IIIa, IIIb, IIIc, IIa, and IIId.

FIG. 3. Pericentric inversion *b* (chromosome III).

detected in association with IIIL "terminal," it has its left break on the region covered by this inversion (fig. 2). It is not known, however, if this break occurred within the inverted or within the Standard segment of the paracentric. In the first case, the pericentric involves ¾ of the left arm; in the second, about ⅝. Only ⅓ of the right arm has been included. It is a clearly asymmetrical inversion.

This pericentric was found in one among the four larvae derived from the collected female and which have been examined. Among 24 F_2 larvae examined, it was also found in only one. In this second case, the heterozygous IIIL "terminal" was also present.

Inversion b (III chromosome).—Highly asymmetrical, with the left break very close to the free end corresponding to the gene arrangement of the "terminal" IIIL inversion. It includes about ⅔ of the right arm and 39/40 of the left arm. It is the longest pericentric found (fig. 3). No paracentric was found associated with it.

Inversion c (III chromosome).—Highly asymmetrical, but not so much as IIIb. With both breaks lying inside the proximal half of both arms, it is the shortest of this group. IIIR break is located not far from the middle of the arm, while the left break is close to the limits of the proximal ⅓ (fig. 4).

In a sample from Antonina (sample 4, table 1), this inversion was found in association with the basal IIIR inversion of Kikkawa (1938). In a Paranaguá sample

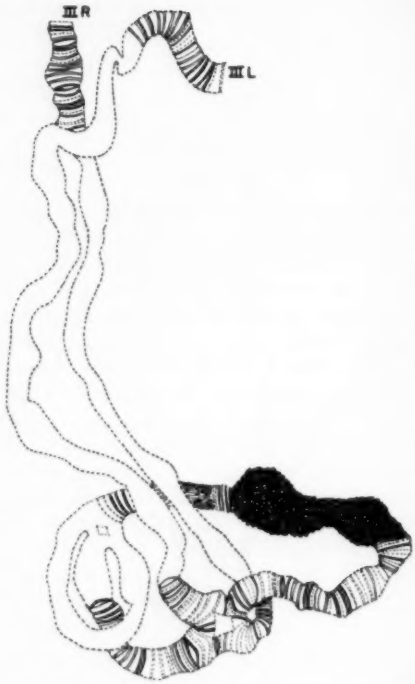


FIG. 4. Pericentric inversion *c* (chromosome III).

(No. 7), it was found together with the median IIR in one larva and with the long subterminal IIL, these paracentrics having been also described by Kikkawa (1938).

Inversion d (III chromosome).—Found associated with basal IIIR and "terminal" IIIL. Very long inversion. The R break is here at its most distal position, including about ⅛ or more of this arm. About 7/10 of the left arm is included, this inversion being a little asymmetrical. No drawing of this inversion is available.

Table 1 presents the incidence of these inversions in the samples studied. It is noteworthy that, while inversions IIa, IIIa, IIIb, and IIId were found only once in the total sample, inversion IIIc has been detected four times in two different localities about 30 km apart in a straight line on the Paranaguá Bay.

TABLE 1. *Incidence of pericentric inversions in chromosomes of* Drosophila ananassae

No.	Localities	Time of collection	Number of individuals examined		Pericentric inversions					%
					Chrom. IIa	Chrom. III				
						a	b	c	d	
1	Caiobá, Paraná[1]	June, 1951	54		0	1	0	0	0	1.85
2	Recife, Pernambuco	July, 1951	35		0	0	1	0	0	2.86
3	Antonina, Paraná	November, 1951	25	87	0	0	0	0	0	2.30
4	Antonina, Paraná	March, 1952	62		0	0	0	2	0	
5	Paranaguá, Paraná	March, 1952	46	192	0	0	0	0	0	1.56
6	Paranaguá, Paraná	September, 1952	67		1	0	0	0	0	
7	Paranaguá, Paraná	October, 1953	49		0	0	0	2	0	
8	Paranaguá, Paraná	September, 1954	30		0	0	0	0	0	
9	Uberlândia, Minas Gerais	March, 1953	29	59	0	0	0	0	1	1.69
10	Uberlândia, Minas Gerais	March, 1954	30		0	0	0	0	0	
	Others		950		0	0	0	0	0	
	Totals		1,377		1	1	1	4	1	8
	Frequencies (%)				0.07	0.07	0.07	0.29	0.07	0.58

[1] The flies were collected a few km from this locality, at a point called Passagem.

Translocation

One larva derived from one of the females belonging to sample No. 9 (see table 1) presented the configuration shown in fig. 5. It is the result of a heterozygous translocation of a segment of IIIR to IIR (cf. schema in fig. 5b). In one cell, the region covered by the translocation was unpaired (fig. 6). The paracentric basal IIIR was also present in the same larva. Another sample collected at the same locality one year later (sample 10) failed to show the same translocation.

This translocation is different from those described by Kikkawa (1938) and Dobzhansky and Dreyfus (1943).

Transpositions (shifts)

Change of position of segments within the same chromosome (transposition) has been detected twice in our total sample, both involving both arms ("heterobrachial shifts," according to White's, 1954, nomenclature). The first example (II chrom.) was seen in one sample composed of 86 individuals collected in Gaspar, Santa Catarina, in June, 1952 (fig. 7). A small sample of 5 individuals from the same locality was analyzed two years later (July, 1954), without showing the shift. The second instance was found in sample 6 (cf. table 1), and involved the III chromosome. The same larva also presented the basal IIIR inversion and the "terminal" IIIL inversion (fig. 8).

FIG. 5. Heterozygous translocation IIR-IIIR. For details, see text.

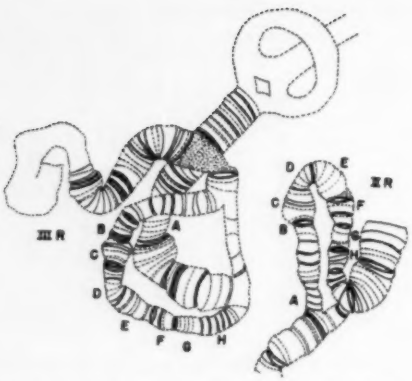


FIG. 6. Heterozygous translocation IIR-IIIR. For details, see text.

Both situations can also be interpreted as the result of two overlapping pericentric inversions (cf. Discussion).

Deletion

One deletion (fig. 9) was detected at the distal region of IIIL in one larva from sample 2 (see table 1). It includes about one dozen bands. Its distal limit lies close to the distal limit of the subterminal paracentric inversion.

Discussion

Pericentric inversions. — Considered as disadvantageous events *per se*, since crossing over within the inversion heterozygotes leads to the production of aneuploid gametes, but known as having played an important role in the phylogeny of a number of species of this genus (Wharton, 1943; Dobzhansky and Pavan, 1943; Patterson and Griffen, 1944; Stone, Griffen, and Patterson, 1942; Burla et al., 1949; Ward, 1949; Freire-Maia et al., 1953), pericentric inversions seem to be, due to their rarity, an unimportant factor now operating on the make-up of *Drosophila* populations. The first example of a pericentric inversion in natural populations of *Drosophila* was found by Miller (1939) in *D. algonquin*. Pericentric inversions were detected also in natural populations of *D.*

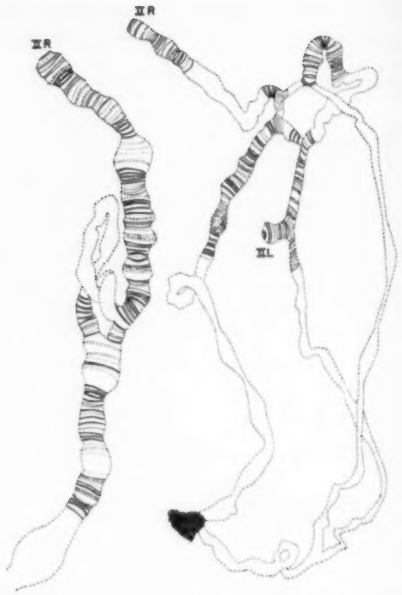


FIG. 7. A transposition (heterobrachial shift) in chromosome II.

robusta by Carson and Stalker (1947), Levitan (1951a, b), and Carson (1958). Here, however, two different inversions were detected (2L–R and 3 L–R), 3L–R being very common in some populations, with frequencies close to 50% in some American localities (Carson, 1958). Homozygotes proved to be fully viable. A fourth possible, but by no means proved, example of pericentric inversion in natural population of *Drosophila* is that responsible for the chromosome difference between the so-called races A and B of *D. kikkawai* (Kikkawa, 1936; Tan, 1942; Tan and Hsu, 1944; Freire-Maia, 1947). Here, however, as the chromosome where the heteromorphy occurs (IV) is largely heterochromatic, the effects on fertility are expected to be entirely or at least largely suppressed.

The number of pericentric inversions found in *D. ananassae* is higher than that detected in all the other *Drosophila* species taken together. It is impressive that some

FIG. 8. A transposition (heterobrachial shift) in chromosome III.

FIG. 9. Deletion in IIL.

of the species most extensively studied, such as *D. pseudoobscura, D. persimilis, D. willistoni,* and *D. funebris,* do not present any example of pericentric inversions. These facts favor the hypothesis that a few species, such as *D. ananassae* and *D. robusta,* probably developed some mechanism capable of "protecting" their populations against the adaptively bad effects of this kind of inversion. Such devices would be capable of annulling, from the very beginning, its intrinsic disadvantages, or counterbalancing them by some special and yet unknown advantage. It is also highly impressive that *D. robusta* is the only species in which a ring chromosome was found in natural populations (Levitan, 1952).

Several interpretations have been proposed for the maintenance of pericentric inversions in natural populations of some *Drosophila* species. Miller (1939) suggested that, as the pericentric inversion he found in *D. algonquin* was associated with two paracentric inversions, these would act as protector devices, since any crossing over would lead to the formation of acentric and dicentric chromatids, which are selectively eliminated, while the non-crossover ones are maintained. None of the pericentric inversions found in *D. ananassae* falls within this possibility.

Alexander (1952) tested two pericentric inversions in *D. melanogaster* for the production of aneuploid gametes, one roughly symmetrical (*Glazed*) and the other highly asymmetical (*Plum*), and found that the asymmetrical one, while slightly longer than the symmetrical, produced fewer

(6.7%) aneuploids than the asymmetrical (9.9%). If the degree of symmetry is responsible for this difference, asymmetrical pericentric inversions would be less disadvantageous than the symmetrical or nearly symmetrical ones. However, none of the pericentric inversions detected in *D. ananassae* (as well as in *D. robusta*) is so extremely asymmetrical that they could have such kind of protection through a yet unknown mechanism.

The size of pericentric inversions can also act as a shielding device capable of eliminating the initial disadvantages, as small inversions, due to the failure in pairing in heterozygotes, are not expected to have crossing over. The inversions we found in *D. ananassae* are, however, sufficiently long to be outside this explanation.

A fourth possibility is the occurrence of pericentric inversions superimposed on rare paracentrics (Patterson and Stone, 1952). No such case exists in *D. ananassae*.

The above suggestions (except that regarding inversion size) do not eliminate the initial disadvantages of the pericentric inversions, but only minimize them. This means that these inversions must always have some initial advantage capable of counterbalancing the initial bad effects. The conclusion is that the smaller the initial disadvantage, the easier it will be for the species to find compensatory mechanisms, and so the higher will be the probability for the inversion to be maintained. It is probable that *D. ananassae* (and *D. robusta*) "discovered" some way of minimizing sufficiently the frequency of crossing over within their pericentric inversions, thus creating new evolutionary possibilities. It is possible that other species have developed such kind of tolerance in previous stages of their evolution, so making possible the changes in chromosome form detected by several authors.

Translocations.—Although translocations have played an important role in the evolution of the genus *Drosophila,* their rarity in natural populations is impressive. As a matter of fact, translocations are known as highly disadvantageous events (cf. Brown, 1940; Wright, 1941).

Only three examples of translocations in natural populations of *Drosophila* species are known, besides the one here reported. It is highly impressive that the first two cases were found also in *D. ananassae* (Kikkawa, 1938; Dobzhansky and Dreyfus, 1943). In this last example it was detected so frequently as twice among the offspring of seven females from a Brazilian locality. The third case, also detected in Brazil, was found in *D. prosaltans* (Dobzhansky and Pavan, 1943; Cavalcanti, 1948). Thus, of the four known cases, three were in *D. ananassae*. It is interesting also that in *D. ananassae* has been found the only really proved case of fixation of a translocation within the karyotype of an animal species (that from the basal region of the X chromosome, where *bobbed* and the nucleolus organizer are situated, to the chromosome IV; cf. Kikkawa, 1937, 1938; Kaufmann, 1936b, 1937). These two facts strengthen the hypothesis that this species has developed a special mechanism capable of neutralizing in some way the selectively bad consequences of the "forbidden" chromosome rearrangements.

Transpositions.—There is general agreement that complex gene arrangements (i.e., arrangements involving three or more breaks) do not arise directly as such, but represent the mere accumulation of simple two-break rearrangements. This hypothesis stems from the consideration that the probability of the simultaneous occurrence of several breaks on the same chromosome is too low to be used as an explanation. The finding of intermediary steps to fill up the complete phylogeny of the complex gene arrangements is direct proof, whereas the first idea is only a statistical probability. The absence of this second datum does not, however, invalidate this kind of interpretation. The postulated intermediary steps are then considered as real "missing links." As a matter of fact, the later discovery of these intermediary steps is a proof of the

fertility and correctness of the working hypothesis under consideration (cf. Dobzhansky and Socolov, 1939; Dobzhansky, 1941).

In some very special cases, however, this working hypothesis may prove to be less probable than the opposite view. Hsu and Liu (1948), for instance, detected a system of three tandem inversions in a natural population of a Chinese species of *Chironomus*, and presented the interpretation that it probably resulted from the simultaneous occurrence of four breaks in the same chromosome arm, rather than from a succession of three inversions, each with its normal two breaks.

The present description of two three-break shifts in two different South Brazilian populations of *D. ananassae* is based on interpretation, not on the fact itself. Both cases could also be explained through the accumulation of two-break rearrangements. The rearrangement detected in chromosome II, for instance, may have arisen from the accumulation of a hypothetic paracentric and two hypothetic pericentrics (x and y), thus resulting from three successive rearrangements with two breaks each: Standard → Pericentric x → Pericentric y → Transposition, with the paracentric occurring between any two successive steps.

The transposition in chromosome III could have a simpler phylogeny. As no paracentric has to be postulated, two pericentrics (x' and y') are capable of explaining the situation: Standard → Pericentric x' → Pericentric y' → Transposition.

Three facts seem to favor the transposition hypothesis rather than the accumulation hypothesis:

1. On the basis of the latter, we must postulate three breaks at the same "point" regarding II-transposition and two breaks at the same point regarding III-transposition. This is rather improbable on the basis of what is known from other species. No "point" has yet been found showing a specially high breakability that would account for a concentration of breaks.

2. Even in *D. ananassae*, pericentrics are not common events likely to occur twice in the same chromosome in order to give two overlapping pericentrics as postulated in the accumulation hypothesis.

3. No intermediate steps between Standard and the complex rearrangements have been found, which would be a proof of the validity of the accumulation hypothesis.

Although we realize the impossibility of deciding between the two interpretations with certainty, it is our impression that, in the present case, the shift hypothesis seems more probable than the opposite view. If, however, we are interested in following the widespread belief that complex rearrangements arise as a consequence of multiple but simple steps, without weighing the pros and cons for each species case, we would only say that four more pericentrics have to be added to the list of table 1. Thus, a total of 9 different pericentrics would have been found 12 times in all. In this case, the number of pericentric inversions in sample 6 from Paranaguá (table 1) would be 3 (one on chromosome II and 2 on chromosome III), and the frequency of pericentrics on the total Paranaguá sample would be 2.60% instead of 1.56%. In Gaspar, Santa Catarina, the frequency would be $2/86 + 5 = 2.20\%$ instead of 0. The total sample of all the populations would be 0.87% instead of 0.58%.

Summary and Conclusions

1. From the analysis of larvae from 1,377 females of *D. ananassae* inseminated in nature, five pericentric inversions, one translocation, two heterobrachial shifts (one inverted) and one deficiency have been detected. This number is higher than that detected in natural populations of all the other *Drosophila* species taken together.

2. One pericentric inversion was found twice in each of two different localities, its total incidence being equal to 0.29%. The others were detected once each. The frequency of the five pericentrics in the whole sample equals 0.58%.

3. The translocation, which involves

the chromosomes II and III, was found only once. It is different from those reported by Kikkawa (1938) and by Dobzhansky and Dreyfus (1943).

4. The two heterobrachial shifts were found in chromosomes II and III. They can be either the result of a three-break direct rearrangement, or of two overlapping pericentric inversions, plus one paracentric in the case in which the transposed segment is inverted. It is concluded that the first hypothesis is more probable.

5. The deficiency (found at the subterminal region of IIL) involves about a dozen bands and was found only once.

6. It is concluded that *D. ananassae* probably developed some special mechanism through which it can retain, in its natural populations, gene arrangements otherwise clearly disadvantageous. This would provide a basis to explain why so many "forbidden" rearrangements are present in their populations. The fact that at least some are relatively common in the phylogeny of a number of other *Drosophila* species suggests that these species developed in the past the type of tolerance now displayed by *D. ananassae*.

Acknowledgments

I am specially indebted to the following friends and colleagues for collecting or helping me collect the samples used in this investigation: Father J. S. Moure, Mr. A. Freire-Maia, Dr. O. Frota-Pessoa, Dr. C. Pavan, Dr. A. B. da Cunha, Miss Therezinha Stancati Loureiro, Mr. I. F. Zanardini, Dr. Claudio Froelich, Dr. José Pellegrino, Dr. José Portugal Filho, Dr. A. R. Cordeiro, Dr. M. E. Drets, and Dr. C. L. Solari. I am also indebted to Mr. A. Freire-Maia, who prepared part of the slides I examined. My thanks are due to Dr. H. L. Carson, for discussions while I was preparing the manuscript and for reading and criticizing it. It is a pleasure to acknowledge Dr. H. M. Miller, Jr., from the Rockefeller Foundation, for his stimulating interest and valuable assistance to the research program of our laboratory.

Literature Cited

Alexander, M. L. 1952. The effect of two pericentric inversions upon crossing over in *Drosophila melanogaster*. Univ. Texas Publ., **5204**: 219–226.

Brown, M. S. 1940. The relation between chiasma formation and disjunction. Univ. Texas Publ., **4032**: 11–64.

Burla, H., A. Brito da Cunha, A. R. Cordeiro, Th. Dobzhansky, C. Malogolowkin, and C. Pavan. 1949. The *willistoni* group of sibling species of *Drosophila*. Evolution, **3**: 300–314.

Carson, H. L. 1958. The population genetics of *Drosophila robusta*. Adv. Gen., **9**: 1–40.

—— and H. D. Stalker. 1947. Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution, **1**: 113–133.

Cavalcanti, A. G. L. 1948. Geographic variation of chromosome structure in *Drosophila prosaltans*. Genetics, **33**: 529–536.

Dobzhansky, Th. 1941. Discovery of a predicted gene arrangement in *Drosophila azteca*. Proc. Nat. Acad. Sci., **27**: 47–50.

Dobzhansky, Th., and A. Dreyfus. 1943. Chromosomal aberrations in Brazilian *Drosophila ananassae*. Proc. Nat. Acad. Sci., **29**: 301–305.

Dobzhansky, Th., and C. Pavan. 1943. Chromosome complements of some South-Brazilian species of *Drosophila*. Proc. Nat. Acad. Sci., **29**: 368–375.

Dobzhansky, Th., and D. Socolov. 1939. Structure and variation of the chromosomes of *Drosophila azteca*. J. Hered., **30**: 3–19.

Freire-Maia, N. 1947. Sôbre os cromosomas de *Drosophila montium*. Bol. Fac. Fil. Ciênc. Let. Univ. S. Paulo, **86**, Biologia Geral, **7**: 3–19.

——. 1953. New data on the incidence of pericentric inversions in Brazilian populations of *D. ananassae*. Dros. Inf. Service, **27**: 91–92.

——. 1954. Pericentric inversions in *Drosophila*. Dros. Inf. Service, **28**: 118–119.

——. 1955. *In*: Discussion. Cold Spring Harb. Symp. Quant. Biol., **20**, p. 270.

Freire-Maia, N., I. F. Zanardini, and A. Freire-Maia. 1953. Chromosome variation in *Drosophila immigrans*. Dusenia, **4**: 303–311.

Hsu, T. C., and T. T. Liu. 1948. Microgeographic analysis of chromosomal variation in a Chinese species of *Chironomus* (Diptera). Evolution, **2**: 49–57.

Kaufmann, B. P. 1936a. A terminal inversion in *Drosophila ananassae*. Proc. Nat. Acad. Sci., **22**: 591–594.

——. 1936b. The chromosomes of *Drosophila ananassae*. Science, **83**: 39.

——. 1937. Morphology of the chromosomes of *Drosophila ananassae*. Cytologia, Fuj. Jub. Vol., pp. 1,043–1,055.

KIKKAWA, H. 1936. Two races of *Drosophila montium* (a preliminary note). Jap. J. Genetics, **12**: 137–142.

——. 1937. The inert chromosomes of *Drosophila ananassae* Doleschall. Cytologia, Fuj. Jub. Vol., **12**: 125–128.

——. 1938. Studies on the genetics and cytology of *Drosophila ananassae*. Genetica, **20**: 458–516.

LEVITAN, M. 1951a. Experiments on chromosomal variability in *Drosophila robusta*. Genetics, **36**: 285–305.

——. 1951b. Selective differences between males and females in *Drosophila robusta*. Am. Nat., **85**: 385–388.

——. 1952. A ring chromosome in wild-caught *Drosophila* (abstract). Genetics, **37**: 600.

MILLER, D. D. 1939. Structure and variation of the chromosomes in *Drosophila algonquin*. Genetics, **24**: 699–708.

PATTERSON, J. T., AND W. S. STONE. 1952. Evolution in the genus *Drosophila*. The Macmillan Co., New York. 610 pp.

PATTERSON, J. T., AND A. B. GRIFFEN. 1944. Relationships of *Drosophila montana* and *D. lacicola* to other members of the virilis group. Univ. Texas Publ., **4445**: 194–211.

SEECOF, R. L. 1957. Cytological Analysis (Section III of a paper by W. S. Stone et al., 1955). Univ. Texas Publ., **5721**: 260–316.

STONE, W. S., A. B. GRIFFEN, AND J. T. PATTERSON. 1942. *Drosophila montana*, a new species of the virilis group. Genetics, **27**: 172.

TAN, C. C. 1942. The nature of the "race differential" chromosome in *Drosophila montium* de Meijere. Sci. Rec., **1**: 178–187.

—— AND T. C. HSU. 1944. On the racial differentiation of *Drosophila montium*. Kwangsi Agriculture, **5**, No. 1 (abstract).

WARD, C. L. 1949. Karyotype variation in *Drosophila*. Univ. Texas Publ., **4920**: 70–79.

WHARTON, L. T. 1943. Analysis of the metaphase and salivary chromosome morphology within the genus *Drosophila*. Univ. Texas Publ., **4313**: 282–319.

WHITE, M. J. D. 1954. Animal cytology and evolution. Cambridge Univ. Press. 454 pp.

WRIGHT, S. 1941. On the probability of fixation of reciprocal translocations. Amer. Nat., **75**: 513–522.

HETEROSIS AND FITNESS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

HAMPTON L. CARSON
Department of Zoology, Washington University, St. Louis, Missouri

Received October 3, 1960

Evolution is a process which results in the genetic adjustment of an interbreeding group of organisms to its environment. The pervading principle in this adjustment is natural selection. Operating under the conditions imposed by the prevailing mutation rates, population sizes and recombination patterns, natural selection builds a gene pool which copes with the particular environmental vicissitudes that the population encounters.

The present paper reports the results of laboratory experiments in which the kinetics of genetic adjustment are subjected to direct study at the level of integration of the sexual population. A set of environmental parameters is arbitrarily chosen, gene pools of various compositions are prepared and then are tested for performance under these conditions. The average size that the population maintains, when equilibrated over many generations by natural selection, is used as the measure of the performance of the group. A control or standard population is always maintained under identical conditions and is measured simultaneously with the experimental population being tested. That population which maintains the larger relative size is judged to be performing better from the overall biological point of view. Such a collective measure is referred to as the relative population fitness.

These experiments, of which preliminary reports have been made in Susman and Carson (1958), Carson (1958 a and b), Bert (1960) and Smathers (1961), show that natural selection in certain populations of *Drosophila melanogaster* favors balanced polymorphism due to autosomal heterosis. This polymorphism develops rapidly and is associated with a pronounced and sudden rise in fitness. Fitness levels are greatest soon after genetic alteration of the base populations, suggesting that simple luxuriant heterosis rather than coadaptive integration is serving fitness. Decline rather than increase of fitness with time further indicates that coadaptation is not involved.

MATERIAL AND METHODS

The material consists of three well-known laboratory stocks of *Drosophila melanogaster*. These are: 1. an ordinary wild-type Oregon-R (No. a4 of Indiana University); 2. a stock (hereafter for convenience called "sesro" which is homozygous for five third-chromosome genes: sepia (*se*), spineless (*ss*), kidney (*k*), sooty (e^s) and rough (*ro*) and 3. a third chromosome stock, Dichaete Stubble claret (D_3 Sb ca_2) balanced over the 3L and 3R Payne inversions.

The system of maintaining populations in vials is modified from that of Buzzati-Traverso (1955). The adult flies of each experimental population ("standing crop") are maintained in a chamber made of an ordinary glass shell vial (95 × 25 mm) which is extended in length by attaching a tube of cellulose acetate of the same dimensions ("supervial") to the mouth of the glass vial by drafting tape. The chamber of the supervial contains a small platform of blotting paper which serves as an additional area in the chamber for flies to rest upon and provides some moisture control. The unit is closed tightly at the end with cotton. The glass vial ("population vial") contains 9.5 cc of a carefully prepared, uniform, cornmeal-Karo-Agar medium prepared exactly according to the directions in Carson (1958b). The surface of the medium is yeasted with 10 mg of Fleishmann's fresh dry yeast, moistened with one drop of distilled water. A strip of absorbent toweling (15 × 75 mm) is doubled and pushed

TABLE 1. *Example of cyclic weekly maintenance and measurement schedule for an experimental population*

Day of week[1]	Operations performed
Saturday	1. Open the population chamber and tape a **FRESH POPULATION VIAL** (No. 1) in position onto the supervial containing the population. 2. Set aside the population vial which was removed from the population. This is the weekly "extra" or "egg sample" vial which is discarded or used for samples. 3. **COLLECT NEWLY-EMERGED FLIES** from all old population vials onto unyeasted medium.
Monday	1. Etherize, **WEIGH AND COUNT YOUNG FLIES** which have been on unyeasted medium. Set aside momentarily. 2. Open the population chamber, ADD the young flies from (1) above directly into the population and tape a **FRESH POPULATION VIAL** (No. 2) in position onto the supervial containing the population. 3. Plug population vial No. 1 tightly and set into a vial rack. It is not opened again until F_1 flies appear, at which time collections from it begin. 4. **COLLECT NEWLY EMERGED FLIES** from all old population vials onto unyeasted medium.
Wednesday	1. Open the population chamber and tape a **FRESH POPULATION VIAL** (No. 3) in position onto the supervial containing the population. 2. Plug population vial No. 2 tightly and set into a vial rack. It is not opened again until F_1 flies appear, at which time collections from it begin. 3. **COLLECT NEWLY EMERGED FLIES** from all old population vials onto unyeasted medium.
Friday	1. Open the population chamber, remove population, etherize lightly and **WEIGH AND COUNT ENTIRE POPULATION.** Set aside momentarily. 2. Etherize, **WEIGH AND COUNT YOUNG FLIES** which have been on unyeasted medium. 3. ADD flies from (2) above to the population and place in a clean supervial. 4. Tape a **FRESH POPULATION VIAL** ("extra") in position onto the supervial containing the population. 5. Plug population vial No. 3 tightly and set with other two from same week in a vial rack. These vials are not opened until F_1 flies appear, at which time collections from them begin. 6 **COLLECT NEWLY EMERGED FLIES** from all old population vials onto unyeasted medium.

[1] Operations are always completed before noon.

down into the food cake to the bottom of the vial.

The founder individuals of a population, consisting of at least 50 flies of each sex, are placed in such a chamber. A new population vial is substituted for the old on a strictly regular change cycle. The vials removed from contact with the adult flies are plugged and set aside until F_1 flies appear. As soon as emergence begins, the young flies are collected and added directly into the chamber together with the older flies. Under these circumstances, the adult population builds up rapidly to a maximum size. After an initial tendency to oscillate, the population comes into equilibrium with the food source in such a way that the weekly additions from the old vials approximately equals the weekly accumulation of dead flies in the chamber. The details of the weekly cycle of operations of an exemplary population are given in table 1. Population vials are always retained until all flies have emerged; thus no artificial selection is made for genes favoring rapid development.

The experiments to be reported here were all begun by first establishing a base pop-

TABLE 2. *List and description of experiments reported in this paper, several forthcoming papers and in Carson 1958b. For derivation of populations and further details, see fig. 1 and text. C = control; E = experimental*

Experimental population No.	Period No.[1]	Designation in Carson 1958b	Base population	Infected with single autosome sets from:
C-1a	1	C-1	se ss k e^s ro	none, control
C-1b	1	–	se ss k e^s ro	none, control
C-1c	1	C-2	se ss k e^s ro	none, control
C-1d	1	C-3	se ss k e^s ro	none, control
C-1e	2	–	se ss k e^s ro	none, control
C-1f	2	–	se ss k e^s ro	none, control
C-1g	3	–	se ss k e^s ro	none, control
C-1h	3	–	se ss k e^s ro	none, control
C-4a	1	C-4	Oregon-R	none, control
C-4b	2	–	Oregon-R	none, control
C-5	1	C-5	Oregon-R	none, control
E-1a	1	E-1	se ss k e^s ro	Oregon-R
E-1b	2	–	se ss k e^s ro	Oregon-R
E-1c	3	–	se ss k e^s ro	Oregon-R
E-1d	3	–	se ss k e^s ro	Oregon-R
E-1e	3	–	se ss k e^s ro	Oregon-R[2]
E-2a	1	E-2	se ss k e^s ro	Oregon-R
E-2b	2	–	se ss k e^s ro	Oregon-R
E-3	2, 3	–	se ss k e^s ro	none[3]
E-4	2, 3	–	se ss k e^s ro	none[3]
E-5	2	–	se ss k e^s ro	Oregon-R, followed by D_3 Sb ca_3
E-6	2	–	se ss k e^s ro	Oregon-R, followed by D_3 Sb ca_3
E-7	2	–	Oregon-R	se ss k e^s ro
E-8	2	–	Oregon-R	se ss k e^s ro
E-9a	2	–	Oregon-R	se ss k e^s ro
E-9b	3	–	Oregon-R	se ss k e^s ro
E-9c	3	–	Oregon-R	se ss k e^s ro
E-9d	3	–	Oregon-R	se ss k e^s ro[2]

[1] Period No. 1: January 1957–April 1958
Period No. 2: May 1958–February 1959
Period No. 3: March 1959–October 1959

[2] Back selection experiments, reported in Smathers, 1961

[3] X-ray experiments, not reported in this paper

ulation of flies from either the sesro or Oregon stocks. After a stated interval, foreign genetic material was introduced into these populations in minimal amounts. Ordinarily this was done by adding to the population in question a single male individual which was an F_1 from a cross between a female chosen at random from the population and a male from the donor stock.

A list of the populations formed in the course of this study is given in table 2, and the derivation of each population with respect to ancestral populations is given in figure 1. The course of the experiments is divided into three time periods: No. 1, January 1957–April 1958; No. 2, May 1958–February 1959; No. 3, March 1959–October 1959. Certain of the populations, e.g. the control population C-1 (fig. 1; table 2), have run continuously throughout the three periods covered by this paper. The same designation number has been retained throughout for a given population but a lower-case letter has been affixed to the number to apply to the various replicates or direct descendents of this population which have not been purposely changed genetically. When a genetic change is made in a population, that population is given an "E" (experimental) number. Replicates or linear descendents of it bear the same number but are distinguished by lower-case letters.

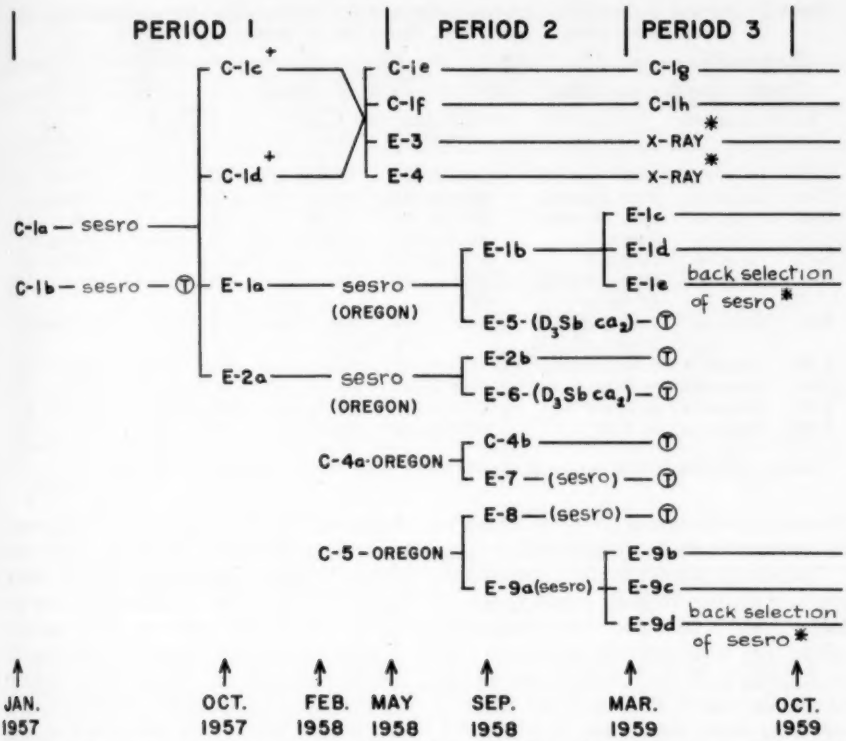


FIG. 1. Derivation of various experimental populations of *Drosophila melanogaster*. The genetic nature of the base population is given: "sesro," "Oregon," etc. Introduced autosome sets are in parentheses. Populations marked by asterisks (*) have been reported in Smathers (1961) or in a forthcoming paper.

Populations were duplicated, when desired, in the following manner. For example, the two control sesro populations C-1a and C-1b were established from stock in January of 1957. In October of 1957, C-1b was terminated and C-1a divided into four parts, C-1e, C-1d, E-1a and E-2a. The flies from the adult population were divided equally among the four new populations. The population vials from which flies were hatching were distributed as evenly as possible among the four replicates. Under these circumstances it takes about seven or eight weeks before the new equilibrium is reached and measurements can be recommenced. Ordinarily, populations were split into two rather than four new populations. On one occasion, two populations were combined and then split into four replicates (C-1d and C-1e, May, 1958: see figure 1.)

As indicated in table 1 (Friday, No. 1) data on the size of adult populations were obtained once a week by etherizing, weighing and counting the adults present in the population chamber. At approximately four-week intervals, the phenotypes of the adult flies in the populations were also recorded.

Two times a week, the young emerging flies were weighed, counted, and added to the population. The phenotypes of these were recorded at approximately 4-week intervals. The total weight (or number) of flies emerging in any one calendar week is referred to as the "production" of the population. In tables 3 and 4, for example,

TABLE 3. *Size and production of experimental populations of* Drosophila melanogaster *during 20 consecutive weeks at equilibrium. Period No. 2: 1958–59 (see fig. 1)*

Population No.	Base population	Single autosome sets introduced from	Population size		Production	
			Mean No. individuals per week	Mean wet weight (mg per week)	Mean No. individuals per week	Mean wet weight (mg per week)
C-1b[1]	se ss ro	none (control)	161.3 ∓ 7.3	94.4 ± 3.3	92.7 ± 7.2	47.2 ± 4.5
C-1e	se ss ro	none (control)	169.0 ± 8.8	104.0 ± 5.5	83.3 ± 5.3	42.3 ± 2.4
C-1f	se ss ro	none (control)	157.7 ± 8.3	99.8 ± 5.7	78.7 ± 5.5	41.3 ± 2.9
E-1b	se ss ro	Oregon-R	478.4 ± 17.4	279.0 ± 9.4	239.4 ± 14.5	128.7 ± 8.2
E-2b	se ss ro	Oregon-R	477.3 ± 11.7	280.5 ± 7.3	239.9 ± 14.7	128.6 ± 7.3
E-5	se ss ro	Oregon-R, then D^a Sb ca^2	471.5 ± 18.6	263.6 ± 9.4	224.6 ± 16.4	119.3 ± 8.7
E-6	se ss ro	Oregon-R, then D^a Sb ca^2	496.5 ± 21.6	283.5 ± 10.4	233.9 ± 19.3	122.4 ± 10.0
C-4b	Oregon-R	none (control)	348.4 ± 8.8	202.3 ± 5.9	162.2 ± 11.6	87.0 ± 5.5
E-7	Oregon-R	se ss ro	376.6 ± 16.6	215.5 ± 8.4	183.2 ± 13.8	98.6 ± 6.9
E-8	Oregon-R	se ss ro	476.5 ± 16.2	278.6 ± 7.1	253.0 ± 13.3	140.5 ± 7.3
E-9a	Oregon-R	se ss ro	467.0 ± 12.0	281.4 ± 7.1	258.7 ± 16.6	145.4 ± 9.5

[1] Based on 17 consecutive weeks at equilibrium; Period No. 1: 1957–58 (see fig. 1).

the mean production is given for a number of consecutive weeks, usually 20.

The weekly population size measurements upon which the 20-week means shown in tables 3 and 4 are based were obtained by taking the total starting population size (e.g., table 1, Friday, No. 3) and adding to this the size of the population which is surviving seven days later (e.g., table 1, Friday, No. 1). This sum was then divided by two.

THE EXPERIMENTS

a. *sesro (Oregon) Populations*

Populations so designated are composed of base populations of sesro into which a haploid set of autosomes from Oregon-R were introduced. These populations are designated as E-1 and E-2. They were started on December 23, 1957, when two of four replicate populations of sesro, which had been running at equilibrium, were infected with single males carrying one set of autosomes from Oregon-R. The results observed over the ensuing 30 weeks (period 1) were given in tabular and graphic form in Carson (1958b) and are shown also in fig. 2. This experiment was continued for a total of about 95 weeks. Data on population size and production of these populations, as well as controls running simultaneously, are given in tables 3 and 4 (E-1, E-2 and their controls C-1e to C-1h).

Population sizes in milligrams wet weight of these two populations, relative to their sesro controls, are summarized graphically

TABLE 4. *Size and production of experimental populations of* Drosophila melanogaster *during 20 consecutive weeks at equilibrium. Period No. 3: 1959 (see fig. 1)*

Population No.	Base population	Single autosome sets introduced from	Population Size		Production	
			Mean No. individuals per week	Mean wet weight (mg per week)	Mean No. individuals per week	Mean wet weight (mg per week)
C-1g	se ss ro	none (control)	132.1 ∓ 3.1	77.6 ∓ 1.7	87.1 ∓ 2.7	49.5 ∓ 1.8
C-1h	se ss ro	none (control)	123.0 ∓ 2.4	70.4 ∓ 1.4	84.7 ∓ 3.9	46.7 ∓ 2.0
E-1c	se ss ro	Oregon-R	342.0 ∓ 11.8	177.9 ∓ 6.7	171.8 ∓ 8.0	84.0 ∓ 3.7
E-1d	se ss ro	Oregon-R	346.6 ∓ 17.8	186.7 ∓ 9.1	170.7 ∓ 10.0	86.0 ∓ 4.5
E-9b	Oregon-R	se ss ro	425.6 ∓ 10.6	234.4 ∓ 5.7	220.9 ∓ 8.1	114.7 ∓ 3.9
E-9c	Oregon-R	se ss ro	446.1 ∓ 12.9	243.4 ∓ 6.7	234.6 ∓ 10.6	122.0 ∓ 4.9

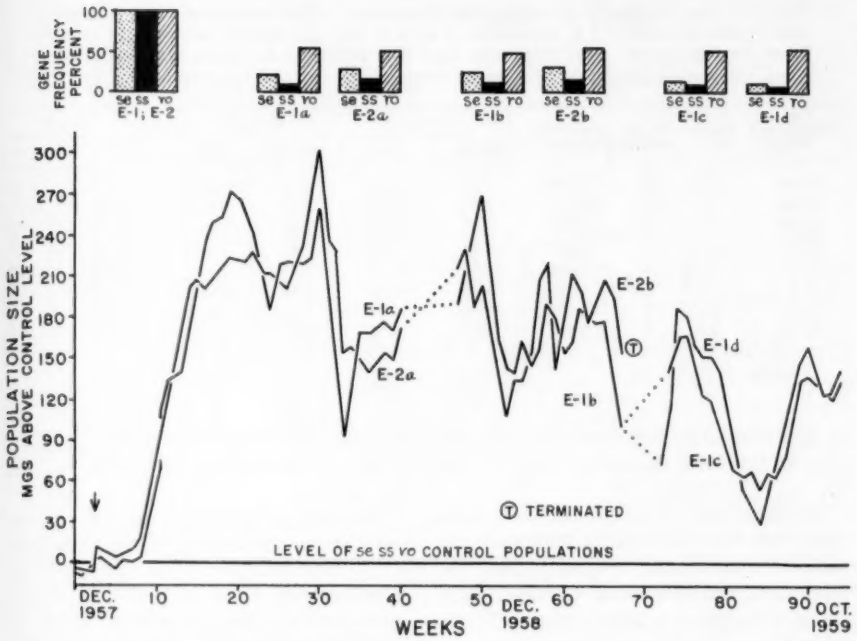


FIG. 2. Size of two sesro (Oregon) populations. These are *se ss ro* base populations to each of which a single set of autosomes from Oregon-R was added (arrow). The histograms show the frequencies of the genes before (extreme left) and during (right) the course of the experiments (see tables 2, 3, and 4).

in fig. 2. In this figure, the mean weekly size levels of the two control populations are represented along a horizontal line parallel to the abscissa and the level of each experimental is represented in terms of the milligrams difference from this control level. After introduction (arrow, fig. 2), population size rises steeply to a level which, in period 1, is 3.45 times that of the control. After about 30 weeks the size of both populations falls to an intermediate level, which, during period 2, is 2.75 times the control. During the third period, approximately weeks 70–90 (fig. 2), the population size falls to a new low level which is 2.47 times that of the control. This means that after 90 weeks the populations had lost about one-quarter of the new size gained following introduction of foreign genes.

Following introduction of the males, there was an immediate drop in the frequency of the marker genes observed in the population. Free recombination was observed. Frequencies of phenotypes of *se*, *ss*, and *ro* have been recorded at regular intervals throughout the experiments; the results of these counts are given in table 5 and are summarized in the histograms given along the top of fig. 2. Estimates of gene frequency have been made by taking the square root of the frequency of the homozygous phenotypes.

In general, after the initial rapid adjustment (see Carson, 1958b), frequencies of the marker genes have been quite stable throughout the course of the experiment. Not only were *se*, *ss*, and *ro* present after 95 weeks but *k* and e^s were also still present in substantial frequencies. Detailed analysis, however, did not encompass these latter two genes. Rough, for instance, fell

TABLE 5. *Gene frequencies in experimental populations of the composition "sesro (Oregon)," that is, ones in which a base population of* se ss ro *flies was infected with one set of autosomes from Oregon-R. Gene frequencies have been calculated by taking the square root of the zygotic frequencies of observed homozygotes. See also histograms in fig. 2*

Population No.	Period No.[1]	No. of samples	Total flies counted	Sepia		Spineless		Rough	
				%se/se	%se	%ss/ss	%ss	%ro/ro	%ro
E-1a	1	12	4,376	4.04	20.11	0.94	9.67	27.99	52.91
E-2a	1	12	4,532	6.53	25.55	2.30	15.16	26.96	51.92
E-1b	2	5	2,501	5.12	22.62	1.39	11.81	21.07	45.90
E-5	2	5	2,562	4.76	21.82	0.47	6.86	22.21	47.13
E-2b	2	5	2,801	10.99	33.15	2.14	14.63	25.06	50.06
E-6	2	5	2,582	9.99	31.62	2.59	16.09	30.75	55.45
E-1c	3	5	1,757	1.99	14.11	0.91	9.54	26.41	51.39
E-1d	3	5	1,823	1.37	11.71	0.55	7.41	26.77	51.74

[1] See fig. 1.

to approximately 50% and has remained close to this level thereafter (table 5; fig. 2).

Although both *se* and *ss* declined somewhat from their high points, both were still present in substantial frequency when the experiment was terminated (*se* was a little above 10% and *ss* a little under 10%; see table 5).

b. *Oregon (sesro) Populations*

Populations so designated are the reciprocal of the former. They are composed of Oregon-R base populations into which a haploid set of autosomes from sesro was introduced. The data for one control and three experimentals are given in tables 3 and 4 (C-4, E-7, E-8, and E-9). Control populations C-4 and C-5 have been previously reported on in Carson (1958b). The population sizes of all these populations are shown graphically in fig. 3. Single males carrying one set of autosomes from sesro were added to populations E-7, E-8 and, E-9a at week 43, in September 1958 (arrow, fig. 3). As this operation consisted of adding a wild-type fly to a wild-type population, it was not possible to see immediately if the infection of the three populations was successful. In populations E-8 and E-9a, however, mutant individuals appeared about three weeks later (see "X" marks in week 46, fig. 3). In population E-7, however, mutant individuals did not appear until after a long lag period of about 10 weeks (see "X" mark in week 63, fig. 3).

Reference to table 3 shows that the control Oregon population (C-4b) maintains a size approximately twice the size of the sesro controls (2.03 in period 2; this is somewhat less than period 1, when the comparable figure was 2.34). In the two experiments where the sesro chromosomes became immediately established (E-8 and E-9a), the population sizes rapidly increased until they were 2.80 (E-8) and 2.83 (E-9a) times the sesro controls. Both of these populations thus well surpass the control Oregon population. Population E-9, carried into period 3 in two replicates (E-9b and E-9c, table 4) showed a slight increase over this original level. E-9b is 3.18 and E-9c is 3.29 times the size of the control sesro populations in period 3. Thus, the introduction of sesro autosomes into Oregon stock produces a population with a relative population fitness about the same as the reciprocal experiment, sesro (Oregon). The Oregon (sesro) experiments were not carried for a long time as the reciprocals were. Therefore, it is not possible to see if a long-term breakdown in relative fitness comparable to that observed in the sesro (Oregon) occurred.

Population E-7, fig. 3, showed a lag period before the marker genes appeared. During this lag, the population showed no

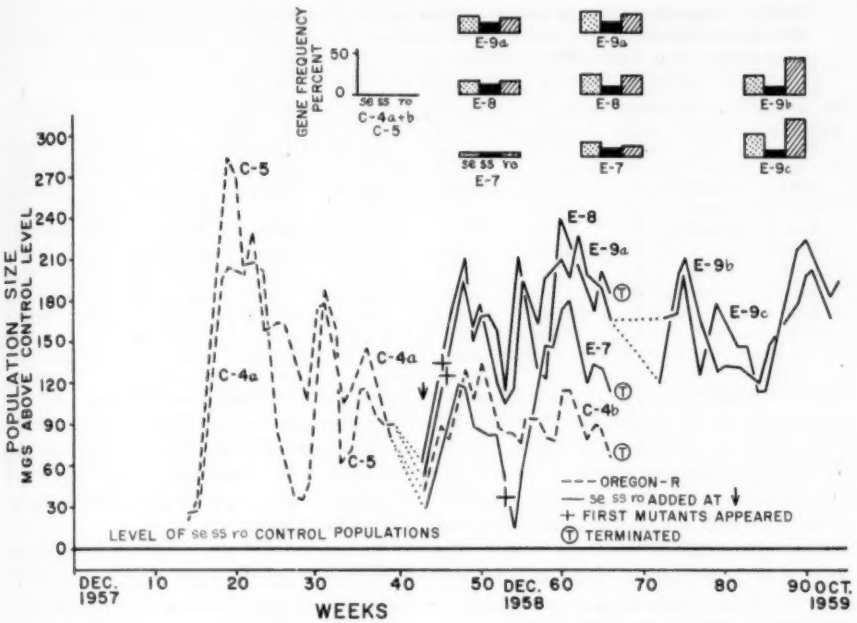


FIG. 3. Size of control Oregon populations (– – – –) and three Oregon (sesro) populations (————). The latter are Oregon base populations into each of which a single set of autosomes from *se ss ro* has been added (arrow). The histograms show the frequencies of the genes during the course of the experiments (see tables 2, 3, and 5).

increase in size, but was, in fact, somewhat below the control. Coincident with the appearance of the mutants, however, a sudden rise in size occurred (weeks 54 to 60, fig. 3) and the average size of the population during period 2, as given in table 3, does not reflect this late change. Although this experiment was terminated in March of 1959, a sample of the population was placed in ordinary laboratory culture in bottles. All five marker genes were still present in this stock at the time of discard at the end of December, 1959.

Once introduction of the marker genes into the Oregon base populations was accomplished, the frequency of each of the three genes *se*, *ss*, and *ro* rose to levels which are very similar to those observed in the reciprocal experiments (table 6 and the histograms in fig. 3). Rough, which reached a frequency of about 50% in experiments E-1 and E-2, was again the most frequent of the markers, but was somewhat less frequent, reaching a level of about 43%. Sepia is next in frequency and spineless is the least frequent; this is the same order as observed in the reciprocal experiments.

c. *sesro* (*Oregon*) (*D*^{s} *Sb ca*^{s}) *Populations*

Populations so designated began as sesro base populations to which were added successively (1) an autosome set from Oregon-R and (2) an autosome set from a laboratory stock having a third chromosome marked with dichaete, stubble, and claret. This latter chromosome included the inversion In3LD. Both dichaete and stubble are homozygous lethal.

Four replicates of sesro (Oregon) populations were established in September, 1958 by dividing populations E-1a and E-2a (see fig. 1). Immediately after division, and before the populations had risen

TABLE 6. *Gene frequencies in experimental populations of the composition "Oregon (sesro)," that is, ones in which a base population of Oregon-R flies was infected with one set of autosomes from* se ss ro *stock. Gene frequencies have been calculated by taking the square root of the zygotic frequency of observed homozygotes. See also histograms in fig. 3*

Population No.	Period No.	No. of samples	Total flies counted	Sepia		Spineless		Rough	
				%se/se	%se	%ss/ss	%ss	%ro/ro	%ro
E-7	2-early	4	1,584	0.19	4.35	0.19	4.35	0.19	4.35
E-7	2-later	4	1,942	2.63	16.22	1.03	10.15	1.65	12.84
E-8	2-early	4	2,146	2.70	16.43	1.44	12.00	2.47	15.72
E-8	2-later	4	2,353	5.14	22.67	1.02	10.09	5.27	22.96
E-9a	2-early	4	2,015	4.12	20.30	1.34	11.58	3.28	18.11
E-9a	2-later	4	2,387	6.24	24.98	1.42	11.92	5.03	22.43
E-9b	3	5	2,269	5.38	23.19	0.97	9.85	17.85	42.25
E-9c	3	4	1,599	7.63	27.62	0.63	7.94	20.14	44.88

again to equilibrium size, individual males carrying a set of autosomes from the D^3 stock and the rest of their chromosomes from the population concerned, were introduced into two of the replicates (populations E-5 and E-6). In both cases, although these males lived for about five days in each population, no D^3 Sb offspring were observed. Accordingly, a second introduction was attempted a month later. Ten virgin females hatching from each population were placed with a single male of the same composition as the above and left for five days in an ordinary culture tube. As soon as many larvae appeared, these ten females and the male were introduced into the respective populations.

Following this procedure, flies showing both dominants appeared in both populations in small numbers. In population E-5, two D^3 Sb flies hatched in the population about 2 weeks after introduction; presumably these were F_1's from the introduced flies. About a month later, one individual carrying D^3 Sb and two carrying D^3 alone hatched, but subsequently no further flies carrying either mutant were seen, and it appears that the genes were eliminated. Attempts to introduce the genes into population E-6 met with greater success. Ten F_1 individuals were observed and subsequently individuals carrying either D^3 or Sb or both were observed regularly. Both genes remained in the population at about 1% until termination of the experiment in March of 1959, a total of about six months. Laboratory cultures derived from this latter population were examined periodically until discarded in December, 1959, and no D^3 or Sb were observed. They were thus apparently eliminated soon after the population was placed in stock.

Reference to table 3 will show that the introduction of these genes and the chromosomes carrying them into the population is not accompanied by any further rise in the relative population fitness.

d. *Survival of Mutant Phenotypes in the Adult Population*

Most of the above populations were regularly examined and the phenotypes counted under the binocular microscope. These counts were made both on flies recently eclosed from the pupa and flies existing in the adult population. In both cases the flies were under continual strong natural selection. Tables 7 and 8 show the frequencies of the three mutant phenotypes in the different populations at eclosion and in the adult population. In almost every case sepia phenotypes are less frequent in the adult populations than at eclosion. In four cases these differences are statistically significant at the levels shown in the tables. Spineless shows the same tendency; the totals for the reciprocal experiments are significant at the 1% level.

On the other hand, flies of a rough phenotype show the opposite tendency, that is, they tend to be more frequent in the adult population than at hatching. It will

TABLE 7. *Survival of mutant homozygotes in sesro (Oregon) populations. Frequency of each phenotype at eclosion from the pupa is compared with the frequency in the adult population*

Population	Total	Per cent se/se	Difference, adult minus eclosion	Per cent ss/ss	Difference, adult minus eclosion	Per cent ro/ro	Difference, adult minus eclosion
E-1a							
eclosion	1,352	4.51		1.26		27.81	
adult	2,848	3.76	−0.75	0.77	−0.49	28.16	+0.35
E-1b							
eclosion	722	4.85		1.39		16.48	
adult	1,779	5.23	+0.38	1.29	−0.10	22.93	+6.45[2]
E-1c							
eclosion	542	2.03		1.11		21.40	
adult	1,215	1.98	−0.05	0.82	−0.29	28.64	+7.24[2]
E-1d							
eclosion	520	2.31		0.77		23.85	
adult	1,303	1.00	−1.31[1]	0.46	−0.31	27.94	+4.09
E-5							
eclosion	597	5.70		0.50		20.27	
adult	1,965	4.48	−1.22[1]	0.46	−0.04	22.80	+2.53
E-2a							
eclosion	1,026	7.12		4.19		29.92	
adult	2,024	6.97	−0.15	1.83	−2.36[2]	29.30	−0.62
E-2b							
eclosion	655	11.6		2.75		22.60	
adult	2,146	10.8	−0.8	1.96	−0.79	25.82	+3.22
E-6							
ecolsion	701	10.98		4.85		27.10	
adult	2,191	9.68	−1.30	1.87	−2.98[2]	31.36	+4.26[1]
Total							
eclosion	6,115	6.20		2.21		24.55	
adult	15,471	5.88	−0.32	1.23	−0.98[2]	27.17	+2.62[2]

[1] χ^2 for the difference indicates a p value less than 0.05 .
[2] χ^2 for the difference indicates a p value less than 0.01 .

be noted, however, that in some individual cases the differences are not very great.

e. *Associations of linked Mutant Markers in the Populations*

All sesro (Oregon) and Oregon (sesro) populations show occasional individuals hatching which are phenotypically sepia-spineless, sepia-rough, or spineless-rough. The question arises whether any of the five linkage markers introduced (*se*, *ss*, *k*, *e*[s] and *ro*) have remained in these populations in non-random association. To test this, over 100 wild-type males were taken from each of four populations (E-1c, E-1d, E-9b, E-9c) and their two third chromosomes tested by crossing to sesro virgin females. Analysis of these and comparable data from a number of other experimental populations will be presented in a later publication.

DISCUSSION

When two genetically different cross-fertilizing populations of the same species are subjected to identical controlled laboratory conditions under natural selection, the population which maintains the larger size may be said to be performing better, from the biological point of view, than the smaller one. The larger population thus may be said to have a collective genetic endowment, or gene pool, which enables it to exploit the substrate provided by the investigator in such a way as to produce a greater biomass than the smaller population. Population size, then, may be used as a measure of the fitness of the mendelian population as a whole. The term *relative*

TABLE 8. *Survival of mutant homozygotes in Oregon (sesro) populations. Frequency of each phenotype at eclosion from the pupa is compared with the frequency in the adult population*

Population	Total	Per cent se/se	Difference, adult minus eclosion	Per cent ss/ss	Difference, adult minus eclosion	Per cent ro/ro	Difference, adult minus eclosion
E-7							
eclosion	685	2.63		1.02		1.72	
adult	1,690	2.13	−0.50	0.89	−0.13	1.30	−0.45
E-8							
eclosion	1,196	5.35		1.84		4.26	
adult	3,303	3.48	−1.87[2]	1.00	−0.84[1]	3.82	−0.44
E-9a							
eclosion	1,242	6.92		2.50		4.99	
adult	3,160	4.62	−2.30[2]	0.95	−1.55[2]	3.99	−1.00
E-9b							
eclosion	709	5.36		0.99		15.37	
adult	1,560	5.38	+0.02	0.96	−0.03	18.97	+3.60[1]
E-9c							
eclosion	538	8.74		0.74		15.61	
adult	1,061	7.07	−1.67	0.57	−0.17	22.43	+6.82[2]
Total							
eclosion	4,370	5.79		1.63		7.28	
adult	10,774	4.23	−1.56[2]	0.92	−0.71[2]	7.48	+0.20

[1] χ^2 for the difference indicates a p value less than 0.05 .
[2] χ^2 for the difference indicates a p value less than 0.01 .

population fitness is suggested for this attribute. The genetic adjustment of different gene pools may be compared in this manner.

Both number of individuals and biomass wet weight have been used in this study as measures of the relative population fitness. The data show, however, that the two modes of measurement are about equally useful because the mean weight per fly does not show sharp reduction as the population increases in number following hybridization. In fact, a slight change of the opposite sort is observed. In most of the experiments, number and weight of flies hatching each week ("production") was also recorded separately. This measure, however, is essentially a part of the overall population size; its usefulness is further limited by the fact that considerable week-to-week variance is encountered.

The relative population fitness bears no relationship to the fitness in the sense of the adaptive value, W (see Dobzhansky, 1951). The latter is a property of an individual genotype and refers to its relative capacity to transmit its genes to the next generation. The relative population fitness is an attribute of a mendelian population measured in laboratory tests under controlled conditions. The measure cannot be applied to natural populations because equilibration of the environment is not possible.

In the present experiments, it has been shown that when a mutant population is infected with a limited amount of genetic material from a wild-type stock, the size of the new hybrid population rises within a few generations to a level well above that of either donor population. This high fitness was maintained for about fifteen generations, after which periodic slight declines were observed. The reciprocal experiment, in which genetic material from the mutant stock is introduced into a wild-type population, a similar result is obtained, although the new population size level is reached more slowly. Nevertheless, populations derived in the two ways ultimately show closely similar properties.

Accompanying the change in size in each population in which third chromosome marker genes have been used, a balanced polymorphism for these genes has devel-

oped. In all experiments, furthermore, each of the five marker genes has been retained in relatively high frequency under the continual, severe natural selection which obtains in these populations. This condition indicates the presence of heterozygous advantage for these gene loci or at least for chromosome sections marked by these genes. The data further suggest that the principal object of selection has been a generally beneficial heterosis.

That this heterosis does not depend to a significant degree on heterotic effects of the precise marker loci themselves is indicated by the work of Smathers (1961). Using replicates of polymorphic populations E-1 and E-9 (E-1e and E-9d, fig. 1), Smathers performed back selection for a monomorphic mutant phenotype while maintaining large numbers of flies so as to minimize the effect of random drift. When the performance of these rederived monomorphic populations was tested simultaneously with the polymorphic and original monomorphic controls, their performance was close to that of the polymorphic populations. Evidently, heterozygosity at these particular loci contributes little, if any, to population fitness. Smathers suggests that polygene blocks of unknown size, closely linked to the markers, may be responsible for the heterosis observed in these experiments.

Heterosis is observed almost immediately and the effect is strongest at the beginning of the experiments, shortly after introduction. Thus, the heterosis functioning here appears to be simple luxuriance rather than populational heterosis (coadaptation) which would require a time-consuming developmental process involving recombination and subsequent selection of recombinant types. This conclusion is further borne out by the results of the experiments of Bert (1960). When inversions were introduced in such a way that recombination is effecively prevented in the population, the heterosis observed is not greatly different from that seen in experiments in which recombination is freely permitted by the cytogenetic situation.

That luxuriant heterosis appears to serve fitness at the beginning of the experiments does not necessarily mean that coadaptation could not follow in later generations. There is, in fact, ample opportunity for the development of coadaptation, especially within populations E-1 and E-2, which ran for nearly two years under conditions of free recombination. During this time, however, the fitness level has declined rather than increased. Thus, if coadaptation did occur in the later generations it must have been effective only in preventing an even greater loss of fitness due to drift in these small populations. It seems likely to the writer, however, that only luxuriant heterosis is operative in these experimental populations.

The slow breakdown of fitness may be explained as due to the loss of heterozygosity because of the effect of inbreeding and random drift in these relatively small populations. According to this idea, first generation hybrids would be expected to display the greatest luxuriance, and as recombination proceeds in the population it may be expected that it will slowly destroy the integrity of blocks of genes having heterotic effects. Thus heterozygosity for a number of chromosome regions would be lost by random drift. In this sense, recombination is destructive of population fitness in relatively small, inbred populations which are exploiting the advantages of luxuriant heterosis.

An experimental population to which foreign genetic material has already been added appears to be easily saturated. The evidence for this comes from those experiments in which attempts were made to introduce genes from the dichaete-stubble stock into a population already carrying a series of mutant genes. These experiments, however, should be looked upon more as pilot experiments than as constituting a fair study of the process of building up of multiple heteroses.

The present experiments permit no de-

cision as to whether the heterosis effects observed are due to dominance, overdominance or to some combination of the two (Crow, 1952). Whatever the most important type of gene action turns out to be, however, these experiments demonstrate that the fitness of the mendelian population under natural selection may be directly served by the condition of heterozygote superiority. This superiority is not a characteristic of F_1 generation individuals only but, if the proper conditions of population size are maintained, can be held by selection over a number of generations. This suggests that luxuriance can indeed function in the adjustment of organisms to their environments and is not confined to the F_1 of artificially-made crosses.

In a recent general article (Carson, 1959), the writer has contrasted two types of genetic adjustment: that in which selection favors the homozygous condition at most loci (homoselection) and that in which selection for heterozygotes (heteroselection) predominates. In the current experiments, heteroselection, with resulting balanced polymorphism, appears to be the major mode of genetic adjustment. This does not appear to represent the acquiring by the population of specific genetically determined features which adapt its members to the special niches presented within the experimental population. Rather, natural selection seems to favor the heterozygous condition because of the superior general vigor with which the heterozygous individuals are endowed.

Phenomena similar to those studied in these experiments apparently occur often in natural populations. Whenever two relatively inbred populations which have been historically separate come into contact without full sexual isolation, events similar to those studied here may follow. Thus local increase in population size and performance may result. The population may spread in a radial fashion, often moving against ecological gradients. A number of examples from natural populations have been cited by Carson (1959), including cases of both intra- and interspecific hybridization. One case may be cited here which may serve as a further example. Wilson and Brown (1958) state that in approximately 1918, a "dark phase" of the fire ant, *Solenopsis saevissima* was introduced into the United States, apparently from southern South America. During the first ten years or so following its introduction it was apparently unable to spread outside of the limits of Mobile, Alabama, where the introduction occurred. About 1930, however, there began a population expansion throughout the southeastern United States which has reached colossal proportions. This event is correlated with an apparent second introduction of a "light phase" of the same species from central South America. It is tempting to suggest that this spread has amounted to an example of adjustment through heteroselection of the type studied experimentally in this paper.

Changes of this kind, however, do not represent major evolutionary adjustment in the sense that the organism acquires and fixes the genetic basis for the conquering of a new ecological niche or niches. If the basis for adjustment is luxuriance with an underlying unfixed genetic condition characterized by balanced polymorphism, this is a type of evolutionary adjustment that may be progressively improved only to a certain point. The population cannot go beyond this by vigor selection alone. New niches may indeed be entered but these are conquered only by virtue of the general adaptability of heterotically buffered organisms, not because an adaptive novelty has been acquired.

Summary

1. A procedure is described for maintaining experimental populations of *Drosophila melanogaster* in vials in such a way that the amount of food and the change cycle is strictly uniform.

2. Measurements were made of the size of various genetically different populations maintained under the same conditions

while under natural selection in the laboratory.

3. Each of two populations homozygous for five third chromosome recessives (sesro) were infected with a single autosome set from Oregon-R stock. Population sizes were measured thereafter for 94 successive weeks. The size of both populations increased nearly three and a half times within about three generations. At the termination of the experiment, the experimentals had declined somewhat, falling to about two and a half times the control size. Reciprocal experiments gave similar increases in population size but the effect occurred more slowly.

4. Correlated with the rise in population size the mutant genes became balanced in the populations. Although the gene frequencies vary somewhat from population to population, rough is always the most frequent (average: 51.4%) sepia next (average: 24.4%) and spineless the least frequent (average: 12.3%). Kidney and sooty were likewise retained in all populations. Attempts to superimpose more third chromosome genes were not successful.

5. When two genetically different populations are tested under these uniform population conditions in the laboratory the one which is able to maintain the greater size, or biomass, is deemed to be performing better biologically under the given conditions. Size thus may be used as a measure of the relative population fitness.

6. High relative fitness appears to be directly correlated with heterozygosity; thus heterosis appears to contribute directly to fitness.

7. The experiments provide no evidence that coadaptation of chromosomes is involved in any of the adjustments that the population makes, although this possibility cannot be excluded. Rather, the evidence supports the view that heterosis is of the simple luxuriant sort. This luxuriance is not confined to the early generations but is persistent over many generations.

Acknowledgments

I am especially grateful to Miss Janette Corn for painstaking and unfailing assistance in the handling of these populations on the very arduous schedule required by the experimental design. I wish also to thank Mrs. Marion Stalker for preparing the figures. The work has been supported by Grant G-7441 from the National Science Foundation.

Literature Cited

Bert, G. R. 1960. Fixed heterozygosity and fitness in *Drosophila melanogaster* populations under strong natural selection. Rec. Gen. Soc. Amer., **29**: 57.

Buzzati-Traverso, A. A. 1955. Evolutionary changes in components of fitness and other polygenic traits in *Drosophila melanogaster* populations. Heredity, **9**: 153–186.

Carson, H. L. 1958a. Increase of fitness in experimental populations following introduction of one haploid set of autosomes. Proc. Xth Intern. Congr. Genet., **II**: 44–45.

——. 1958b. Increase in fitness in experimental populations resulting from heterosis. Proc. Nat. Acad. Sci., **44**: 1,136–1,141.

——. 1959. Genetic conditions which promote or retard the formation of species. Cold Spring Harb. Symp. Quant. Biol., **24**: 87–105.

Crow, J. F. 1952. Dominance and overdominance. *In*: Heterosis, Ed. by J. W. Gowen, Iowa State College Press, Ames. 552 pp.

Dobzhansky, Th. 1951. Genetics and the Origin of Species. 3rd ed. Columbia University Press, New York. 364 pp.

Smathers, Katrina M. 1961. The contribution of heterozygosity at certain gene loci to fitness of laboratory populations of *Drosophila melanogaster*. Am. Nat., **95**: 27–37.

Susman, M., and H. L. Carson. 1958. Development of balanced polymorphism in laboratory populations of *Drosophila melanogaster*. Am. Nat., **92**: 359–364.

Wilson, E. O., and W. L. Brown, Jr. 1958. Recent changes in the introduced population of the fire ant *Solenopsis saevissima* (Fr. Smith). Evolution, **12**: 211–218.

OUTLINES OF EVOLUTION IN THE PSEUDOGAMOUS *RANUNCULUS AURICOMUS* GROUP IN FINLAND

GUNNAR MARKLUND AND ARNE ROUSI

Department of Botany, University of Helsinki/Helsingfors, and Department of Botany, University of Turku, Finland

Received March 28, 1961

Ranunculus auricomus L. with its nearest relatives forms a clearly distinctive natural group in the genus. The group as a whole is distributed over most of Europe, extending into Siberia and Caucasia in the east and into Greenland in the west, but within the group some forms, e.g., *R. cassubicus* L. and *R. monophyllus* Ovcz. have a more eastern distribution; others, e.g., *R. auricomus* L., are more western. The species complex presents a number of taxonomic problems, mainly owing to pseudogamous reproduction, a mode of apomixis where pollination is a prerequisite for seed formation although the egg cell develops parthenogenetically. As a rule, in pseudogamy, fertilization of central nucleus is needed before endosperm formation occurs and seeds can be formed (Rutishauser, 1959). Pseudogamy in the *Ranunculus auricomus* group was first discovered by Rozanova (1932), and independently by Koch (1933) and Sørensen (1938). In the crossing experiments of these authors the progeny always turned out to be maternal, but seeds were never formed without pollination. These results were supported by the cytological work of Häfliger (1943), and especially Rutishauser (1954a, b). The mode of reproduction gives the group an exceptional position within the genus because, as far as is known, no species of *Ranunculus* outside of this group reproduces regularly by apomixis. In fact, the whole family Ranunculaceae seems otherwise to be almost devoid of apomictic forms (cf. Nygren, 1954).

For more than twenty years, the senior author has been working with the problems of taxonomy and distribution of the Finnish representatives of the *Ranunculus auricomus* group. A herbarium material of around 32,000 sheets has been accumulated, which gives a rather good picture of the various kinds of biotypes existing in Finland as well as of their distribution and ecological nature. The constancy of morphological characters has been tested by some, admittedly few, uniform garden experiments, using both transplanted individuals and progeny grown from seed. The junior author has been concerned with the cytology of the group. The present paper is his attempt to correlate the taxonomic, phytogeographical, and cytological results in order to obtain an idea of the evolutionary mechanisms operating in the group to cause its obvious diversity.

TAXONOMIC TREATMENT

Considerable divergence exists in the practice of naming apomictic biotypes. Whereas in sexually reproducing plant groups the amount of possible gene exchange between populations provides a criterion of their genetic relationship and helps in determining species boundaries, in apomictic groups this criterion is totally missing. Because of their mode of reproduction, the apomictic biotypes exchange genes only to the extent determined by the possible facultativeness of apomixis. The high degree of constancy displayed within each apomictic biotype, and the very obvious variational gaps between biotypes, give the impression of a group of clearly distinct and taxonomically "good" species. It is no wonder that many taxonomists have treated apomictic groups by giving a specific name to each biotype. The number of species in apomictic groups becomes, however, immensely large by this method, and knowing them becomes a matter of very few well-trained specialists. Also, as Stebbins (1950) points out, several such "species" may arise

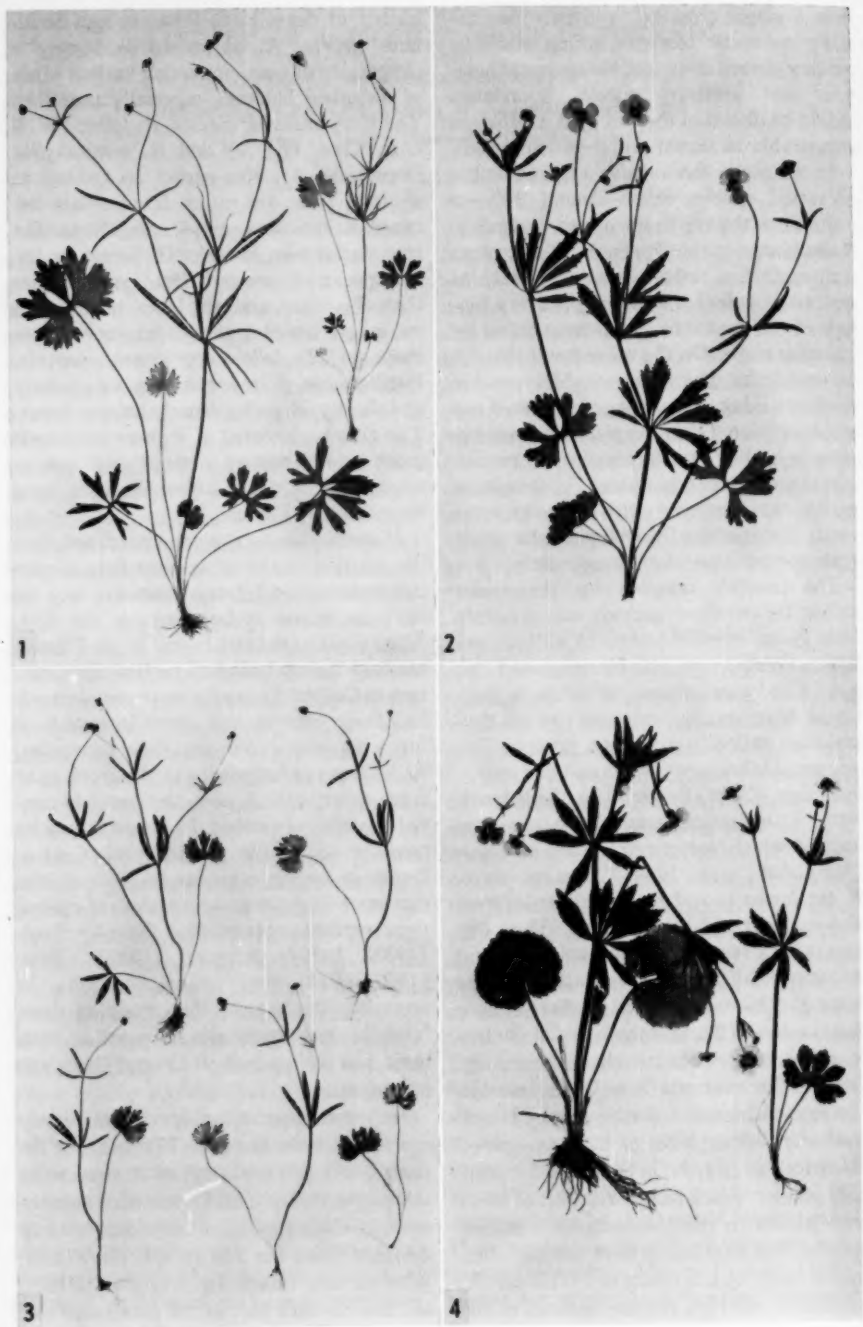
from a single cross between two facultatively apomictic biotypes, which would be a rather absurd situation. He suggests therefore that arbitrary species boundaries should be drawn in the way that a situation comparable to sexual groups would arise.

In the genus *Ranunculus* a large number of sexual species exist—around 300—in addition to the relatively narrow apomictic *R. auricomus* group. Particularly in a genus such as this, a rather distorted picture of systematic values would arise if every biotype of the apomictic group were called by a species name. On the other hand there is no doubt that the apomictic biotypes are worth studying and naming. As pointed out by Gustafsson (1947), apomictic taxa, because of their constancy, offer certain advantages, e.g., in plant geographical studies, as compared with sexual species which are continually changing the genotypic composition of their populations.

The practice adopted by the senior author, for the above reasons, was to refrain from giving specific names to all biotypes but to combine several biotypes into one species and give subspecific names to individual biotypes, i.e., microspecies as they are often called in apomictic groups. This was possible because the Finnish biotypes—more than 400 in all—could be divided with rather little arbitrariness into four groups, each of which was given a specific name (Marklund, 1954, 1960). Two of these, *R. auricomus* L. and *R. cassubicus* L., were already described by Linnaeus. They display very conspicuous morphological and ecological differences, the latter species being also more eastern in its distribution. *R. cassubicus* (fig. 4) is the larger of the two in every respect, being characterized by large, entire, reniform base leaves in addition to some bladeless sheaths and by broad, markedly dentate lobes of the stem leaves. *R. auricomus* (fig. 1), in turn, has considerably smaller, lobed base leaves, all of them with lamina in the Fennoscandian microspecies. The stem leaves have narrow lobes, mostly with entire margins. Whereas *R. cassubicus* has high requirements as to the quality of the soil and grows in lush deciduous groves, *R. auricomus* is largely a companion of man, preferring various kinds of disturbed habitats, especially meadows. The two remaining species, *R. fallax* (W. & Gr.) Kern. (fig. 2) and *R. monophyllus* Ovcz. (fig. 3), also earlier recognized as separate taxa, are rather intermediate between *R. auricomus* and *R. cassubicus*. The intermediacy is, however, different in the two species. *R. monophyllus* is more tender than *R. fallax* and the lobes of its stem leaves are little, if at all, dentate whereas those of *R. fallax* are clearly dentate. Furthermore, *R. monophyllus* has sparsely and shortly, *R. fallax* densely hairy achenes. The distribution area of *R. monophyllus* is much more eastern, comprising mostly northern Asia. Only a few biotypes have extended into Finland.

It should be once more underlined that the division of the group into four species is a somewhat arbitrary treatment and we have no reason to be dogmatic about it. Although it works rather well in the Finnish material it may very well be that the situation in Central Europe is more complicated, i.e., there may be too many intermediate forms impossible to fit into these categories. As long as our knowledge of the group is as fragmentary as it is now, this problem cannot be definitely solved. In future it may be possible to decide whether all Central European microspecies can be grouped into corresponding categories instead of calling them separate species as is done by Koch (1933, 1939), Schwarz (1949), Haas (1952, 1954), and Jasiewicz (1956). It seems for the present that grouping them into the four categories is possible with most, but perhaps not all Central European microspecies.

In some cases a subspecies obviously consists of more than one biotype. For instance, a large population of *R. auricomus* subsp. *glaucescens* Markl. was once encountered in which a group of individuals clearly deviated from the rest of the population. Whereas this subspecies, like most others, has flowers with part of the petals aborted,


1
2
3
4

the deviating group consisted of plants with perfect petals only. Because no other differences could be seen, the most logical explanation is that this deviating group of plants was a result of a gene mutation. It is clear that such slightly deviating biotypes should not be called different subspecies. Usually it is not difficult to distinguish between these minor forms, obviously resulting from gene mutations, and the real microspecies which strongly give an impression of new recombinations of genes.

Chromosome Numbers

Chromosome numbers of 44 subspecies, representing all four collective species, were determined by the junior author (Rousi, 1956). Of the biotypes studied, 42 were tetraploid ($2n = 32$) and 2 were pentaploid ($2n = 40$). The tetraploid number seems to be by far the most common in other areas, too. Häfliger (1943) counted the chromosome numbers of 17 Swiss forms, 14 of which were tetraploid, one diploid, one pentaploid, and one hexaploid. Rutishauser (1954a) reports chromosome counts of 7 more microspecies of Swiss origin, all tetraploid. *R. cassubicifolius* W. Koch is the diploid found by Häfliger and, according to Rutishauser (1954a, 1960), it reproduces sexually. Langlet (1932), Larter (1932), Kurita (1955), and Löve and Löve (1956) report the tetraploid number only, Bruun (1932) both the tetraploid and hexaploid number. Böcher (1938a, b) found a tetraploid strain from Denmark and a diploid one from Greenland. This diploid form is *R. auricomus* var. *glabrata* Lynge (= *R. Lyngei* H. Smith). According to Nannfeldt (1947) it also occurs in Novaya Zemlya and in a few localities in northern Scandinavia.

The great uniformity of the chromosome numbers is noteworthy when the situation in other apomictic groups is compared. The heavy concentration upon the tetraploid number and the absence of triploids as well as aneuploids seem surprising when one takes into consideration the possibilities of apomixis in enabling reproduction of biotypes with unbalanced chromosome numbers. As pointed out by Rutishauser (1959), the absence or scarcity of triploids in pseudogamous plant groups may be a result of the mode of endosperm development in these plants. Rutishauser's (1954a, b) highly interesting experiments on the development of embryo and endosperm in the *Ranunculus auricomus* group indicate that in 98–99% of the cases fertilization of the central nucleus is a prerequisite for formation of endosperm, that this fertilization is usually double in being performed by both of the two generative pollen tube nuclei, and, finally, that the embryo does not develop if fertilization of central nucleus and development of endosperm has not occurred. As shown by Rutishauser (1954a, 1959), a sterility barrier exists between the diploid and tetraploid biotypes in the *Ranunculus auricomus* group, caused by the endosperm breaking down in reciprocal crosses. Obviously the endosperm does not tolerate much disharmony in its chromosomal constitution in spite of the fact that it is a short-lived nutritive tissue. As a result, the situation in respect to chromosome number in the pseudogamous *Ranunculi* resembles the situation in a typical sexual rather than in a typical apomictic species complex.

Meiosis

In autonomously apomictic plants, meiosis always displays a number of irregularities. The reason for this is twofold: firstly, most apomicts are of hybrid origin and secondly, natural selection does not eliminate chromosome rearrangements and other factors causing disturbances as it does in sexual plants where the products of meiosis are genetically important. The pseudogamous plants are somewhat intermediate between sexuals and autonomous apomicts.

Figs. 1–4. Representatives of the four collective species of the *Ranunculus auricomus* group. Fig. 1. *R. auricomus* subsp. *angustisectus* Markl. Fig. 2. *R. fallax* subsp. *euanthemus* Markl. Fig. 3. *R. monophyllus* subsp. *sychnodon* Markl. Fig. 4. *R. cassubicus* subsp. *macranthemus* Markl.

It seems to be a rule in pseudogamous plants that the endosperm develops only after fertilization of central nucleus (cf. Rutishauser, 1959). Thus the sexual phenomena are partly retained in this particular mode of apomixis. As a consequence, the meiotic products on the male side are necessary in development whereas the female meiosis either leads to degenerating cells (in apospory) or is modified to the extent of being non-reductional (in diplospory). Even the male gametes, however, do not take part in the formation of a new individual but only in the formation of the endosperm which presumably is not quite as dependent on a harmonious chromosome and gene combination as a whole plant. In pseudogamous reproduction meiotic disturbances are thus filtered out to some extent, but most probably less than in sexual reproduction.

Microsporogenesis of the *Ranunculus auricomus* group displays a large number of different irregularities (Rousi, 1956). All pollen mother cells of a certain loculus may even die as early as the meiotic prophase, the chromatin first forming droplets and then disintegrating into a deeply stained pycnotic mass. Orientation of the bivalents in metaphase I is somewhat irregular, resulting in a poor plate formation. High numbers of univalents are formed. In four tetraploid microspecies analyzed, they constitute 17–31% of all metaphase configurations. Multivalents are also formed, their incidence in the same microspecies being 9–13% of all configurations. Groups of three and groups of four are about equally common, and in addition there are groups of five, six, and even more chromosomes in the tetraploid strains. Their occurrence strongly indicates structural heterozygosity, as does the occurrence of complicated asymmetrical configurations as well as bridge and fragment formations. In anaphase I laggards are very common, the lagging univalents often dividing at this stage. At the tetrad stage, some of the laggards form micronuclei, but some of them apparently have been included in the main nuclei because the number of micronuclei always is smaller than the number of laggards. In the second meiotic division metaphase plates are often seen which contain abnormal chromosome numbers.

In all probability, therefore, aneuploid pollen grains arise quite frequently. The fact that no aneuploid endosperms were found in the large material investigated by Rutishauser (1954a) suggests that the aneuploid pollen grains seldom, perhaps never, are able to take part in the endosperm formation. Whether this is a result of an inability of aneuploid pollen grains to germinate or of the inviability of an aneuploid endosperm is uncertain. The morphologically poor appearance and scanty germination of the pollen (Rousi, 1956) indicates the first alternative.

The quality of the pollen varied greatly in the different microspecies studied, being poorest in *R. auricomus* subsp. *oligandrus* Markl. Interestingly enough, this microspecies has even a strongly reduced number of anthers. The extremely poor pollen formation in this subspecies would seem to be very disadvantageous for a pseudogamous plant. Nevertheless it is one of the most successful and common Finnish subspecies of the group, growing especially in strongly man-influenced habitats. It is yet to be determined whether, in fact, here is a case of autonomous apomixis or whether this subspecies is dependent on other subspecies in its vicinity as a source of pollen.

Compared with other pseudogamous plants, in which meiosis has been studied, the *Ranunculus auricomus* group has a high amount of meiotic disturbances (Rousi, 1956). Especially its structural heterozygosity is much more pronounced if compared with other pseudogamous groups. Yet the few experiments made by the junior author (Rousi, 1956) suggest pseudogamous reproduction to be the rule in the Finnish microspecies also. It seems highly probable that most of the meiotic irregularities in this group are a result of the hybrid character of the biotypes. An alternative explanation would be an accumula-

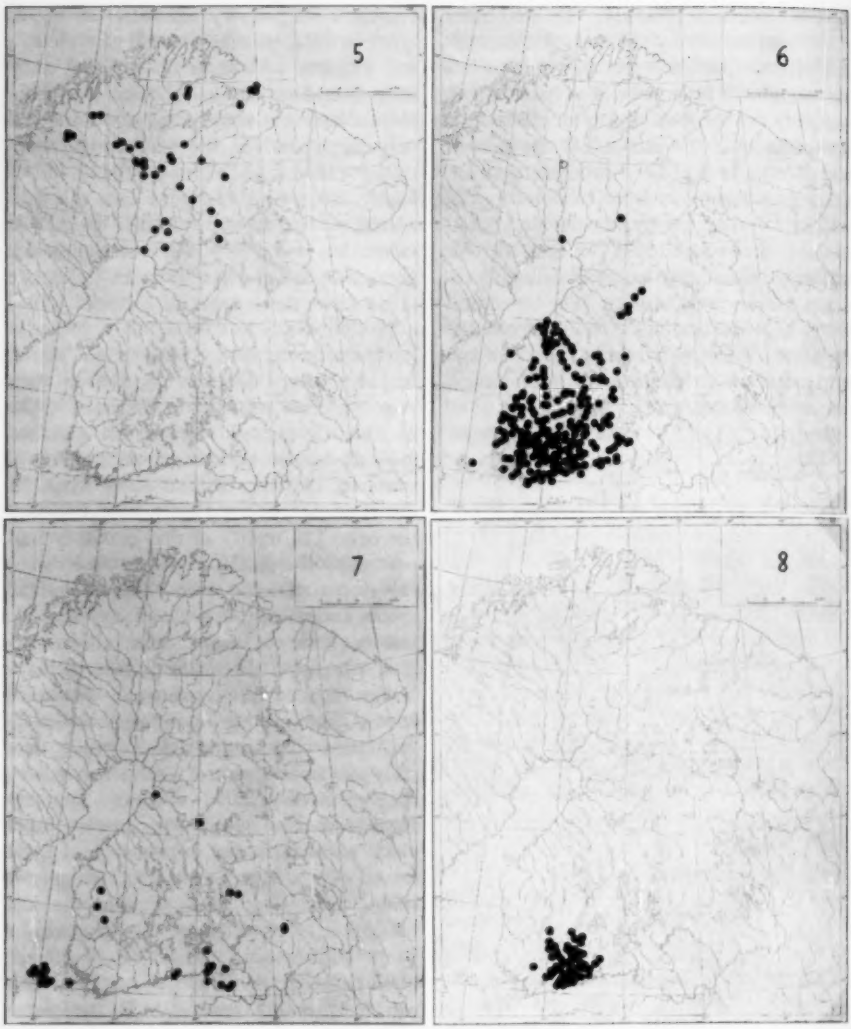
tion of structural heterozygosity and other disturbing factors by the lack of a controlling effect of sexual reproduction. This latter argument may provide a partial but certainly not the total reason for the meiotic disturbances in the group. For the sake of comparison it may be mentioned that another pseudogamous plant, *Rubus caesius*, which is a widespread and common allotetraploid, shows an almost perfectly regular meiosis without any accumulated disturbances (Gustafsson, 1942). The structural heterozygosity and the other meiotic disturbances therefore strongly suggest a hybrid origin of the members of the *Ranunculus auricomus* group.

Geographical Distribution

Apomictic plants have certain advantages in geobotanical studies as compared with sexually reproducing plants. Because of the genetic constancy of apomictic biotypes, their age and distribution history can sometimes be traced quite accurately. For example, *Ranunculus Lyngei* H. Smith (= *R. auricomus* var. *glabrata* Lynge) occurs according to Nannfeldt (1947) in Novaya Zemlya, in a few localities in northern Scandinavia, and in eastern Greenland. These areas have not been united by a land bridge since the interglacial period before the widest distribution of the glaciers. As Nannfeldt points out, *R. Lyngei* must have arisen before that period. It should be noted, however, that this particular taxon is diploid and unknown as to its reproductive method, as emphasized by Jørgensen, Sørensen, and Westergaard (1958).

Among the Finnish representatives of the *Ranunculus auricomus* group, *R. auricomus* subsp. *calvescens* H. Smith has geobotanically a solitary position. It is very definitely northern in its distribution (fig. 5) and, as far as is known, the only subspecies widely spread north of 68° latitude in Finland. Although it usually grows in meadows around villages, it also occurs in very remote areas far from any human influence, e.g., in groves along creeks in the Finnish Lapland and above timberline in some Swedish fjelds. Apparently these wild areas represent its primary habitats, the occurrence in villages being a result of a secondary invasion in human times. Like many other Scandinavian fjeld species, it is probably a very old member of the flora and may have survived the glaciers in unglaciated refugia (Marklund, 1954). It is highly interesting, therefore, that this subspecies is pentaploid (Rousi, 1956). It seems thus that a very long evolution in this group has taken place even in the arctic areas where the number of biotypes is now quite limited.

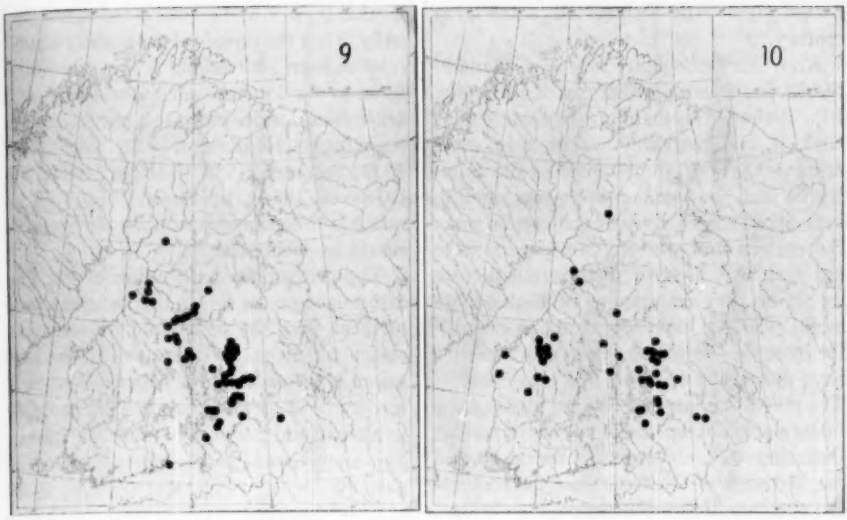
R. auricomus subsp. *glaucescens* Markl. has an entirely different distribution area (fig. 6). This tetraploid subspecies is the one most commonly met in the Helsinki area. It is also very common in most of southern Finland. On the other hand, its distribution area has a sharp border toward the east. The reality of this border is supported by the fact that plenty of *R. auricomus* material has been collected by the senior author east of it (cf. figs. 7 and 9), but there has not been a sign of this particular subspecies. The same is true with the border toward the southwest. The subspecies is almost missing in the whole southwestern archipelago which, being botanically the richest part of Finland, has been an area of very active collecting. The only locality in the Åland area consists of a small population and is probably of quite recent origin. Most of our southern species have migrated to Finland after the last glaciation, either from Sweden through the southwestern archipelago or from the southeast via the Karelian Isthmus. This obviously is not true with *R. auricomus* subsp. *glaucescens*, which is now missing in both areas mentioned although these areas should provide it with plenty of suitable habitats. Instead, it seems highly probable that it has migrated from Estonia across the Gulf of Finland. This microspecies, like most others belonging to the collective species *R. auricomus*, grows mostly in meadows and is therefore dependent on man's action, meadows in Finland being generally a result of human influence.



FIGS. 5–8. Distribution of some representatives of the *Ranunculus auricomus* group in Finland and some adjacent areas. FIG. 5. *R. auricomus* subsp. *calvescens* H. Smith. FIG. 6. *R. auricomus* subsp. *glaucescens* Markl. FIG. 7. *R. auricomus* subsp. *amblyodon* Markl. FIG. 8. *R. fallax* subsp. *mendax* Markl.

Therefore it must have widened its distribution area together with the colonization of Finland. Apparently, however, its spreading has been very slow because large, densely populated areas in eastern Finland and in the southwestern archipelago still await its invasion. It is apparently impossible to explain the limits of its distribution area as a result of any climatic factor.

The distribution map of *R. auricomus* subsp. *glaucescens*, as well as those of other taxa of this group, indicate slow dispersal, a character which obviously is correlated with the structure of the fruit. The achenes



FIGS. 9–10. Distribution maps (continued). FIG. 9. *Ranunculus auricomus* subsp. *lepidus* Markl. FIG. 10. *R. auricomus* subsp. *Collanderi* Markl.

of these plants are rather clumsy and without any special structures for effective dissemination. The difference is quite apparent if the distribution maps of certain apomictic *Taraxaca* are examined. Some of them must have been quite recent immigrants, in many cases more recent than the establishment of grassland cultivation in Finland (Marklund, 1940). Nevertheless, even some of these recent immigrants have occupied a much more extensive area in Finland than any of the members of the *Ranunculus auricomus* group. At least a partial reason for this probably is the pappus of the *Taraxacum* achenes which enables them to spread much more rapidly than the *Ranunculi*.

R. auricomus subsp. *amblyodon* Markl. (fig. 7) is an example of a subspecies with a southern distribution but an entirely different distribution history if compared with subsp. *glaucescens*. Because of its common occurrence in the southwestern archipelago and in the Karelian Isthmus and the big gap between, it seems obvious that this plant immigrated into Finland after the Ice Age via two routes, i.e., the southwestern archipelago and the Karelian Isthmus.

The tetraploid *R. fallax* subsp. *mendax* Markl. has a very clearly defined and rather limited distribution area, but it is quite common within this area (fig. 8). Its distribution picture seems to be partly determined by ecological factors, partly by a slow dispersal. Like most other subspecies of *R. fallax* and all subspecies of *R. cassubicus* this microspecies is restricted to grassy, lush groves. In Finland these kinds of plant communities are confined to rather limited areas. Because this subspecies practically never occurs as a follower of man it has not had the chance to spread out from the grove area it now occupies, although apparently suitable habitats exist outside of this area, too.

R. auricomus subsp. *lepidus* Markl., also a tetraploid, has a very definitely eastern distribution with a peculiar extension of the distribution area in the northwest to Kajaani and even as far as to the Oulu area at the Gulf of Bothnia (fig. 9). The extension to Oulu may be correlated with the fact that rather lively traffic routes have con-

nected Oulu with Kajaani since the 17th century.

Also *R. auricomus* subsp. *Collanderi* Markl. has a very peculiar distribution (fig. 10). Although its main area is eastern Finland, it is remarkably common in some areas in the westernmost part of the country, in fact, too common to be just recently introduced there. Even if the gap between the eastern and western occurrences were not real, this kind of distribution picture would be very uncommon in Finland. It might possibly have something to do with the intensive colonizing of the inhabitants of Savo during the middle of the 16th century. The people of Savo, inhabiting some of the eastern areas of ssp. *Collanderi*, migrated at that time actively towards the west and reached some of the areas close to the western outposts of ssp. *Collanderi*.

There is a surprisingly large number of subspecies of the *Ranunculus auricomus* group which are extremely endemic. There are several subspecies that, as far as is known, consist of a single clone in a certain meadow. They do not seem to have been introduced from abroad because their localities often are very remote from traffic routes or centers of population. They certainly are not relics, either, because their habitats in most cases are temporary and disturbed rather than of relic nature, meadows in Finland being, with a few exceptions, a result of the human influence. The only satisfactory explanation for the endemic occurrence of such microspecies is a relatively recent postglacial origin *in situ*.

Origin of the Diversity

We have seen that more than 400 apomictic microspecies of the *Ranunculus auricomus* group occur in Finland, most of them —42 of the 44 studied cytologically—being tetraploid. The morphological diversity of the group is spectacular, and made still more pronounced by the discontinuity of the variation. Probably no one of the known Finnish microspecies reproduces sexually, so constantly uniform are they all. Also the meiosis of the strains studied was so irregular that they cannot regularly reproduce sexually. Thus the diploid *Ranunculus cassubicifolius* from Switzerland is the only sexual form so far known. All experiments with other microspecies indicate a pseudogamous reproduction (Rozanova, 1932; Koch, 1933, 1939; Sørensen, 1938; Häfliger, 1943; Rutishauser, 1954a, b; Rousi, 1956). In no case has it even appeared that the apomixis would be facultative.

The geographic distribution of the Finnish microspecies shows that whereas some of them, even the pentaploid *R. auricomus* subsp. *calvescens*, are very old, others have arisen quite recently. The main source of the diversity of the group is most probably hybridization, perhaps between *auricomus*-like and *cassubicus*-like sexual types (cf. also Rozanova, 1932; Rutishauser, 1960) and/or between facultatively apomictic forms. Backcrossing presumably has given rise to a large number of new biotypes, some of which have been a starting point for new, well-adapted apomictic biotypes. The whole group gives now an impression of an enormous hybrid swarm, ranging from *auricomus*-like types to *cassubicus*-like types and displaying a wealth of intermediate forms (fig. 11). In the various microspecies the characters of *R. cassubicus* on the one hand and the characters of *R. auricomus* on the other hand have an obvious, although not absolute, tendency to stick together. According to Anderson (1953) a character association like this in a group is strong evidence for past hybridization as a source of diversity. In the *Ranunculus auricomus* group, because it causes a somewhat linear kind of variation, it also helps in arbitrarily dividing the biotypes into the four groups with species names *R. auricomus*, *R. fallax*, *R. monophyllus*, and *R. cassubicus*.

As mentioned earlier, some of the microspecies have a distribution which definitely indicates a quite recent postglacial origin. This is, however, difficult to explain, because no sexual or facultatively apomictic forms are known among the Finnish representatives of the group. Various possibili-

FIG. 11. Lobes of stem leaves of 12 microspecies, displaying typical *Ranunculus auricomus* forms, typical *R. cassubicus* forms, and a series of intermediate forms. Beginning from upper left, five *R. auricomus* subspecies, three *R. fallax* subspecies, and four *R. cassubicus* subspecies.

ties for an origin of these recent forms are, therefore, worth discussion.

New biotypes may, of course, arise through gene mutations. Even though we must never exclude this possibility, it seems likely that gene mutations seldom give immediate rise to new forms in an essentially tetraploid, apomictic plant group. Firstly, the great majority of gene mutations are recessive and should therefore be present fourfold before a phenotypic effect would appear in a tetraploid plant. The microspecies of the *Ranunculus auricomus* group are admittedly of hybrid origin and should probably be called allopolyploids, but it is nevertheless likely that considerable gene duplication exists in the different genomes which have taken part in the hybridization. Secondly, the lack of sexual reproduction and gene recombination means that the possibilities of a recessive mutation becoming fourfold or even twofold in one individual is greatly reduced. Furthermore, the morphological differences characterizing the various microspecies, the most recent as well as the older ones, are always numerous and appear in entirely different ways in different parts of the plants. Although the effect of most genes is known to be pleiotropic, one is inclined to consider differences as great as these to be a result of gene recombination rather than a single gene effect.

Although differences between the microspecies give an impression of being caused by several genes, forms are occasionally encountered which most easily could be interpreted as products of a single gene mutation. For example, as previously mentioned, plants with perfect petals sometimes occur as a distinct group in a population which otherwise consists of individuals with most of the petals aborted. This partial abortion of petals is characteristic of many species of the group. This kind of mutation was also found by Koch (1939) and, as Koch showed, the progeny raised from seeds of this individual remained constant as to the petal characteristic.

Chromosome rearrangements might be considered as a possible source of diversity in an apomictic group. The limitations of this factor are, however, at least as great as of the gene mutations when one takes into

consideration polyploidy and the lack of recombination. Although chromosome rearrangements cannot be considered as a direct way of origin of new apomictic forms, they most probably played an important role in the differentiation of the sexual predecessors of the group. This is shown by the large amount of structural heterozygosity which manifests itself in the meiosis of the apomicts.

Changes in chromosome number have been of very limited importance in calling forth variation in the *Ranunculus auricomus* group. The great majority of all microspecies, both old ones and the most recent endemic ones, are tetraploid ($2n = 32$). The step to tetraploidy has undoubtedly been important in the evolution of the group. Apomixis would presumably not have arisen on the diploid level and the group would now be less polyform and less widespread. Obviously the tetraploid number is optimal in the group, and very few deviations from it have been successful in evolution. Otherwise it would be difficult to explain, for example, the lack of hexaploid forms in our material. Hexaploid embryos very probably arise by an occasional fertilization of an unreduced, i.e., normal, egg cell with a normal sperm nucleus. This is especially obvious when we remember that sperm nuclei always enter the embryo sac and cause single or double fertilization of the central cell, the egg cell staying undivided until the developing endosperm induces division (Rutishauser, 1954a, b). The two pentaploids in our material have most probably arisen either by fertilization of an unreduced egg cell by a sperm nucleus of a diploid individual, or by hybridization between a hexaploid and a tetraploid form. In the case of the northern pentaploid *R. auricomus* subsp. *calvescens*, a diploid parent might have been *R. Lyngei* whose distribution area also includes Northern Scandinavia. A diploid or hexaploid is not found in the vicinity of the distribution area of the other pentaploid, *R. cassubicus* subsp. *dispar* Markl., the origin of which therefore remains completely unsolved.

Autosegregation is a phenomenon which sometimes causes diversity in the progeny of an apomict without fertilization or change of chromosome number (Gustafsson, 1947). It is probably caused by some remains of meiotic phenomena which still may occur in the formation of the embryo sac in diplosporous plants. Because embryo sac formation in the *Ranunculus auricomus* group is aposporous (Häfliger, 1943), this phenomenon can scarcely be considered possible in any phase of its development.

The only remaining satisfactory explanation for the origin of the new microspecies is the incidental occurrence of sexual phenomena in the apomicts. Although all experiments so far indicate an obligate rather than facultative apomixis, one has to remember that nature has an overwhelmingly larger material and longer time to experiment with than can be reached in any artificial circumstances. Even an extremely low incidence of sexual reproduction may be enough in the long run to produce occasional successful recombinants. Apomicts without any possibility for occasional sexual recombination seem, furthermore, to be much rarer than was once supposed. There has even been some doubt as to the existence of obligate apomixis in its strictest sense (cf. Clausen, 1954).

For several reasons it seems most likely that hybridization, which has given rise to the new forms, has occurred between tetraploid apomictic types. If there were a sexual form in Finland it should be much more variable than the apomictic microspecies. The variation is, however, equally narrow in all microspecies studied, indicating that all Finnish forms of this group reproduce at least mainly by apomixis. If some diploid sexual form, like the Swiss *R. cassubicifolius*, nevertheless existed in Finland, it alone would not suffice to explain the origin of new tetraploid forms. Triploid forms have not been found so far and, furthermore, the experiments of Rutishauser (1954b, 1959) revealed sterility barriers between the diploid *R. cassubicifolius* and the tetraploid apomicts. Successful hybridization would

therefore have to involve either two diploid forms with subsequent polyploidization or two tetraploid, most likely facultatively apomictic forms. Although the first kind of hybridization has probably occurred in earlier periods of the evolution in the group, the second kind must be responsible for a great amount of new forms, in any case those which have arisen in the most recent time. The large amount of structural heterozygosity in the youngest as well as in the older microspecies points in the same direction. A recent polyploidization product should not show much structural heterozygosity if meiotic pairing is preferential.

A good example of a very recent evolution within the group is provided by a population which the senior author came upon in South Finland in the parish of Nurmijärvi. On the terrace of the river Palojoki, within a length of ca. 100 meters, four entirely new microspecies were found. This population, as it could be called, gave a strong impression of quite recent hybridization. On the same place the tetraploid *R. auricomus* subsp. *acutiusculus* Markl. was growing and, significantly enough, one of the new microspecies resembled it very much. Otherwise all of the four were morphologically intermediate between *auricomus* and *fallax* types in that the lobes of the stem leaves were dentate but the bladeless sheaths at the base were lacking. The locality falls into the area of frequent occurrence of the tetraploid *R. fallax* subsp. *mendax*. Although it was not found in the immediate vicinity of the population described, it seems possible that it has been one parent of the four new hybrid types.

Summary

The present paper is a discussion of the taxonomic, geobotanical, and cytological investigations of the pseudogamous *Ranunculus auricomus* group. Main attention is given to the evolutionary conclusions which can be drawn from the various lines of investigation. The great polymorphism of the group is explained mainly on the basis of interspecific hybridization. Whereas it appears that originally the crossing occurred on the diploid level, it has more recently occurred to a large extent on the tetraploid level. Crossing between the two levels is apparently hindered by a sterility barrier. New tetraploid biotypes are shown to have arisen even up to the present time, the most probable explanation for their origin being a recent crossing between existing tetraploid, facultatively apomictic biotypes. Although it has not been possible to prove experimentally the facultativeness of apomixis in the group, the occurrence of new, apparently recombinant, biotypes can scarcely be explained without an assumption that the apomicts still have some traces of sexuality.

Literature Cited

Anderson, E. 1953. Introgressive hybridization. Biol. Rev., **28**: 280–307.

Bruun, H. G. 1932. Cytological studies in *Primula*. Symb. Bot. Upsal., **1** (1): 1–239.

Böcher, T. W. 1938a. Biological distributional types in the flora of Greenland. Medd. om Grönl., **106** (2): 1–339.

——. 1938b. Cytological studies in the genus *Ranunculus*. Dansk Bot. Arkiv, **9** (4): 1–33.

Clausen, J. 1954. Partial apomixis as an equilibrium system in evolution. Proc. 9th Internat. Congr. Gen. Caryologia **6**, suppl.: 469–479.

Gustafsson, Å. 1942. The origin and properties of the European blackberry flora. Hereditas, **28**: 249–277.

——. 1947. Apomixis in higher plants. III. Biotype and species formation. Lunds Univ. Årsskr. N.F. avd. 2, **43** (12): 181–372.

Haas, A. 1952. Neue süddeutsche Arten aus dem Formenkreis des *Ranunculus auricomus* L. Ber. Bayerisch. Bot. Gesellsch., **29**: 5–12.

——. 1954. Neuer Beitrag zur Kenntnis des Formenkreises von *Ranunculus auricomus* L. in Süddeutschland. Ber. Bayerisch. Bot. Gesellsch., **30**: 27–32.

Häfliger, E. 1943. Zytologisch-embryologische Untersuchungen pseudogamer Ranunkeln der *Auricomus*-Gruppe. Ber. Schweiz. Bot. Gesellsch., **53**: 318–382.

Jasiewicz, A. 1956. De Ranunculis e circulo Auricomi Owcz. in regione Cracoviensi nec non in Carpatorum parte boreali crescentibus. Fragm. Florist. et Geobot. Ann., **2** (1): 62–110.

Jørgensen, C. A., Th. Sørensen, and M. Westergaard. 1958. The flowering plants of Greenland. A taxonomical and cytological survey. Biol. Skr. Dan. Vid. Selsk., **9** (4): 1–172.

Koch, W. 1933. Schweizerische Arten aus der

Verwandtschaft des *Ranunculus auricomus* L. Ber. Schweiz. Bot. Gesellsch., **42**: 740–753.

——. 1939. Zweiter Beitrag zur Kenntnis des Formenkreises von *Ranunculus auricomus* L. Ber. Schweiz. Bot. Gesellsch., **49**: 541–554.

KURITA, M. 1955. Cytological studies in Ranunculaceae I. Bot. Mag. Tokyo, **68**: 94–97.

LANGLET, O. 1932. Über Chromosomenverhältnisse und Systematik Ranunculaceae. Sv. Bot. Tidskr., **26**: 381–400.

LARTER, L. N. H. 1932. Chromosome variation and behaviour in *Ranunculus* L. J. Genet., **26**: 255–283.

LÖVE, A., AND DORIS LÖVE. 1956. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburg., **20**: 65–290.

MARKLUND, G. 1940. Die *Taraxacum*-Flora Nylands. Acta Bot. Fenn., **26**: 1–187.

——. 1954. Fröbildning utan befruktning och därmed sammanhängande mångformighet hos växter. Soc. Scient. Fenn. Årsb., **31** B(3): 1–23.

——. 1960. Der *Ranunculus auricomus* -Komplex (in Finland). I. Diagnosen und Fundortslisten einiger Sippen des *R. auricomus* L. *coll.* (*s. str.*). Soc. F. Fl. Fenn. Flora Fennica, **3**: 1–128.

NANNFELDT, J. A. 1947. Några synpunkter på den skandinaviska fjällflorans ålder. K. Vetenskapssoc. Årsb., **1947**: 51–85.

NYGREN, A. 1954. Apomixis in the Angiosperms. Bot. Rev., **20**: 577–649.

ROUSI, A. 1956. Cytotaxonomy and reproduction in the apomictic *Ranunculus auricomus* group. Ann. Soc. Bot. Vanamo, **29** (2): 1–64.

ROZANOVA, M. 1932. Versuch einer analytischen Monographie der conspecies *Ranunculus auricomus* Korsh. Trav. Inst. Sci. Nat. Peterhof., **8**: 139–148 (Zusammenfassung).

RUTISHAUSER, A. 1954a. Die Entwicklungserregung des Endosperms bei pseudogamen *Ranunculus*arten. Mitt. Naturforsch. Ges. Schaffhausen, **25**: 1–45.

——. 1954b. Entwicklungserregung der Eizelle bei pseudogamen Arten der Gattung *Ranunculus*. Bull. Schweiz. Akad. Med. Wiss., **10**: 491–512.

——. 1959. Pseudogamous reproduction and evolution. Proc. 9th Internat. Bot. Congr., vol. 2: 337.

——. 1960. Untersuchungen über die Evolution pseudogamer Arten. Ber. Schweiz. Bot. Gesellsch., **70**: 113–125.

SCHWARZ, O. 1949. Beiträge zur Kenntnis kritischer Formenkreise im Gebiete der Flora von Thüringen. IV. *Ranunculus* ser. *Auricomi*. Mitt. Thüring. Bot. Ges., **1**: 120–143.

STEBBINS, G. L., JR. 1950. Variation and evolution in plants. New York.

SØRENSEN, TH. 1938. Ranunkelstudier II. Om variation og nedarvningsforhold hos *Ranunculus auricomus* L. Bot. Tidskr., **44**: 433–438.

STUDIES ON EUROPEAN LAMPREYS

GIUSEPPE S. J. ZANANDREA
Institute of Zoology, University of Milan, Italy

Received March 3, 1961

The purpose of the present work is to summarize the results of studies by the author (1940–1959) on lampreys, of Italy and of some other parts of Europe, on which the author has been engaged for the last 20 years, at the Institute of Comparative Anatomy of the University of Bologna (1940–1955), at the Institute of Zoology of the University of Padua (1956–1959), and at the Institute of Zoology of the University of Milan. Except where otherwise specified, the material referred to below has been examined by the writer, and most of it has been collected by himself or specifically for his studies.

LIFE-HISTORY STUDIES OF THE ITALIAN BROOK LAMPREY, *LAMPETRA ZANANDREAI*

The author's studies of the life history of brook lampreys in Italy have been based on *Lampetra zanandrei* Vladykov (1955), except for the materials from the river Sarno (near Naples) and from Sweden, which belong to *Lampetra planeri* (Bloch). Except as otherwise indicated, the data refer to this newly described species. Prior to 1955 the present writer referred all Italian brook lampreys to *L. planeri*.

Conclusions on analyses of length and weight. — In determining lengths and weights it was necessary to examine many specimens from many streams. More than 8,000 ammocoetes, 150 in metamorphosis, and about 1,500 adults have been examined. Ammocoetes of brook lampreys reach lengths and weights greater than those of adults. This is due to the fact that the brook lamprey does not feed after metamorphosis. The lengths and weights both of ammocoetes and of adults differ in different streams, and sometimes in different parts of the same stream, as shown in fig. 1. These variations are apparently caused mostly by abundance or scarcity of nutrition, and by differences in temperature. The size variations in the brook lampreys of Europe do not form latitudinal clines, as they may do in the river lampreys (see below).

The author's initial study of the growth pattern in *Lampetra zanandreai*, begun in 1940, utilized about 2,000 specimens collected near Treviso (northern Italy). The specimens were divided into four groups: ammocoetes without guanophores, ammocoetes with guanophores, ammocoetes kept without food in running water, and metamorphosed lampreys. The relationship between length and weight was expressed by the formula $W = k\,L^n$, where W is weight, L is length, k a constant. The exponent n was taken to be 3 in the early work, but more recently it has been found that the value of n is somewhat different in *L. zanandreai* and in *L. fluviatilis*. The length–weight relationship is expressed in the brook lamprey by a parabola of the third degree.

The parabolae are coincident and the constants are very similar for all ammocoetes considered separately from adults; the parabolae are not coincident and the constants are very different when the ammocoetes and the adult brook lampreys are considered together.

Duration of larval stage.—Authors do not agree on the problem of the duration of the larval stage in lampreys. The prevailing opinion is that the duration is from 3–6 years (Okkelberg, 1921, 1922; Hubbs, 1925; Knowles, 1941; Schultz, 1930; Ivanova-Berg, 1931; Chappuis, 1939; Applegate, 1950; Hardisty, 1944, 1951; Dendy and Scott, 1953).

It was necessary to define the factors that bear on the problem: (1) extent of the water area in which the fish are caught, in order to assure similar conditions of nutri-

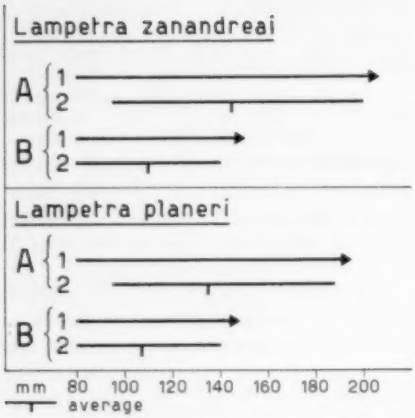


FIG. 1. Differences in length of Italian brook lamprey, *Lampetra zanandreai*, and European brook lamprey, *L. planeri*, depending possibly on nutrition or on variations of temperature during the seasons of year.

A = specimens of greater length; B = specimens of shorter length. 1 = maximum length of ammocoetes; 2 = range of metamorphosed lampreys.

tion; (2) number of ammocoetes caught in a limited period of time.

In order to analyze this problem 1,130 ammocoetes and 30 metamorphosed lampreys without obvious sexual differences were caught in Musoncello Brook, tributary to the Dese River (near Castelfranco, Treviso, Italy), from November 10 to December 10, 1936, along a distance of one kilometer. The ammocoetes in a length–frequency distribution show 4 peaks, the last of which includes the ammocoetes with guanophores. All the adults in a single catch are comprised in one peak; they all belong to lampreys of the same year of life. Similarly, the ammocoetes with guanophores probably belong to the same year, whereas the other ammocoetes belong to distinct years of life. In fact, only the reproduction occurs periodically. It is to be remembered that in the Musoncello Brook the spawning season is very limited (January–February).

Another collection was made in Dese River, over a stretch 2 kilometers long, above the mouth of Musoncello Brook, from December 10 to 20, 1936. Of the 585 ammocoetes caught very few were small. The 60 adults collected with the ammocoetes were completely metamorphosed. The conclusions were the same as for the Musoncello specimens: the ammocoetes showed 4 peaks in their size distribution.

Positive results were also obtained from 208 ammocoetes caught on one day (December 29, 1953) in Goretto Brook, a small tributary of the Po near Turin. In this zone, one kilometer long, some metamorphosed lampreys were also found, but here no ammocoetes of the first year were caught, simply because the fisherman could not catch them.

Thus the brook lampreys of northern Italy live four years and a half in the larval stage, but only half a year in the adult stage after metamorphosis. In arriving at these estimates the writer cannot decide if the ammocoetes with guanophores are in the "rest phase," of which Leach (1951) and several other authors have written.

Sexual maturity and reduction of size.—Previous authors have pointed out that the lampreys exhibit marked differences in secondary sex characters, and have demonstrated that there is a shortening in size in the parasitic species, *L. fluviatilis*, and also in *P. marinus*, between the end of the feeding stage and sexual maturity. Cotronei (1927) believed that this phenomenon could not be demonstrated in the brook lamprey, because metamorphosis and sexual maturity are coincident.

The specimens caught in all life-cycle stages in Treviso waters demonstrated that metamorphosis takes place in the period from August to October, and that spawning occurs in January and February. These two events thus occur at very distinct times. From the completion of metamorphosis to sexual maturity the brook lamprey *L. zanandreai*, as well as the parasitic species, decreases in length. These conclusions were reached both from a comparison of the size range of specimens already metamorphosed, but without secondary dimorphism, with the size range of fully mature lampreys, and from serial measurements of specimens caught at the end of the metamorphosis

TABLE 1. *Life habits and distribution of European species of lamprey*

	Genus	Species	Life	Distribution
(A)	*Lampreys with only the form feeding after metamorphosis*			
(1)	*Petromyzon*	*marinus*	Parasitic; migrating	North Atlantic and Mediterranean tributaries
(2)	*Caspiomyzon*	*wagneri*	Feeding, but not parasitically[1]	Tributaries of Caspian Sea
(B)	*Lampreys with paired forms*			
(3)	*Lampetra*	*fluviatilis*	Parasitic; migrating	European tributaries of Atlantic and of Mediterranean, except Adriatic
(4)	*Lampetra*	*planeri*	Nonparasitic	Same as 3, and two upper Danube tributaries
(5)	*Lampetra*	*j. japonica*	Parasitic; migrating	Northeastern tributaries of Eurasia
(6)	*Lampetra*	*j. kessleri*	Nonparasitic	Same as 5, but lives alone in north-central Siberia
(7)	*Eudontomyzon*	*d. danfordi*	Parasitic; nonmigrating	Tributaries of Theiss (Danube)
(8)	*Eudontomyzon*	*d. vladykovi*	Nonparasitic	Same as 7, and in Austrian, Czechoslovakian, and Jugoslavian tributaries of Danube
(9)	*Eudontomyzon*	*mariae*[2]	Nonparasitic	Lower Danubian and all Russian tributaries of the Black Sea
(C)	*Lamprey with the nonparasitic species only*			
(10)	*Lampetra*	*zanandreai*	Nonparasitic	Tributaries of northern plain of Italy

[1] According to Berg (1931), who did not explain how this lamprey feeds nonparasitically.
[2] According to Berg (1931) it is possible that this species also has a parasitic member, but the author has not yet found such specimens among those he examined (see text).

stage and kept in running water until they became sexually mature.

In other Italian waters brook lampreys metamorphose in the period from August to October as they do in Musoncello Brook and Dese River, but they may spawn from January to June.

This research has comfirmed the conclusion that in brook lampreys, as well as in the parasitic ones, secondary sexual characters appear at the end of sexual maturity, and negates the idea, assumed by some authors until recently, that *L. fluviatilis* and *L. planeri* differ in respect to whether these characters appear or not. This older opinion stemmed from the circumstance that *L. fluviatilis* is caught principally during the spawning runs, when the secondary sexual characters are not yet developed, whereas the brook lampreys are caught almost exclusively on the spawning grounds, when the sexual features are always present and obvious.

Sex ratio, body proportions, and coloration.—Of the 1,314 specimens of brook lampreys examined for sex ratio (among the 1,415 caught until 1951), 777 were males and 537 females. Here the males were predominant (59%), but the sex ratio is not constant in the various periods of life. In the adults, which have not yet developed the secondary sexual characters, the number of males is similar to that of the females, but in the spawning season the males that are caught always outnumber the females. The same conclusions are also valid for the brook lampreys caught in the following years. Perhaps the males live longer than the females, but the apparently altered sex ratio may result from other circumstances.

As for differences in size, these studies point out that when metamorphosis is just complete, the females exceed the males in length and weight. When sexual maturity is reached, however, the females no longer exceed the males in length and weight; at times the males are the longer and the heavier.

The methods used in determining the body proportions were the same as those employed by Hubbs and Trautman (1937) for *Ichthyomyzon* and by Vladykov (1949 and 1955) for many species of lampreys. The length of the head, of the branchial re-

gion, and of the trunk and tail, the diameter of the oral disc and the eyes, and the height of the second dorsal fin were measured. With these measurements relative indices were obtained, which are very significant in studies of speciation and of the various stages in the life cycle.

Sexual dimorphism is comfirmed by certain measurements (diameter of oral disc, height of second dorsal fin, and length of head). The lengths of the trunk and tail are very similar in the two sexes in the transformed lampreys that are still immature. At sexual maturity the males have shorter trunks and longer tails than the females. The relatively longer abdomen of the females is presumably an adaptation for increased fecundity (just before spawning the females are greatly distended with the ripened ova). Brook lampreys of Treviso were found to contain 1,790 to 1,904 eggs (mean, 1,850).

One case of xanthochroism was noted in *Lampetra zanandreai,* caught near Lonigo (Vicenza) and another in *L. planeri* caught near Pompeii (Napoli). Festa (1900) had previously noted such a color variant in the first species, from near Turin.

Many cases of melanism were observed in the lampreys caught near Lonigo (Vicenza). Consequently, this phenomenon characterizes the Italian brook lamprey, as well as the river lamprey, *L. fluviatilis* (Berg, 1948).

Throughout Italy the metamorphosed brook lampreys in life are silvery on both inferior-lateral and ventral sides. On the back and on the superior-lateral side they are generally blue-black before attaining the spawning condition, but are yellowish when spawning. In many specimens it is possible to judge only from the yellowish or blackish coloration whether they are in spawning condition or not.

Systematics and Distribution of Lampreys in Italy

Information on lampreys in Italy is given in works on the Italian fauna in general and on the ichthyological fauna of all Italy or districts of this country. Special studies of lampreys have also been published, but have been based on material from particular streams, e.g., the Tiber, the Arno, and the Sarno Brook (Napoli).

There are no reports based on the direct examination of lampreys, either recently caught in many localities or preserved in Italy and in other European museums. Thanks to the help of the directors of many museums and other scientific institutions, the author has been able to complete a study of this type.

Two species of brook lamprey in Italy.— Vladykov (1955) found, from comparative examination of many specimens of brook lampreys caught in various localities of northwestern Europe, that the specimens sent to him by the author from Lonigo and from Pavia represented an undescribed species, which he kindly named *L. zanandreai.* But Vladykov examined only 15 adults from Lonigo and 20 ammocoetes from Pavia. As can be deduced from the titles of his articles published in 1956, the author first thought that the form described by Vladykov should be regarded as only a subspecies.

Making use of many specimens that served for the earlier studies, and also of many living specimens that are now being obtained for this and other studies, the author has been able to examine extensively the problem of the species of brook lampreys in Italy. The specimens examined included 1,200 of *L. zanadreai* from 37 localities of 14 provinces of northern Italy, from Turin to Gorizia, and 250 of *L. planeri,* from 15 localities not only in the Sarno River, but also in the Tiber, Garigliano, Volturno, and Sele (Salerno); also some from Sweden.

From these studies it is possible to conclude that: (1) all brook lampreys caught in the waters of the north Italian plain have the characters on which Vladykov founded the species *L. zanandreai,* i. e., number and disposition of teeth on oral disc, number of trunk myomeres, coloration of ammocoetes, and body proportions; (2) the characters are very distinct from those of *L. planeri* of Italy and other countries of Europe; (3) in

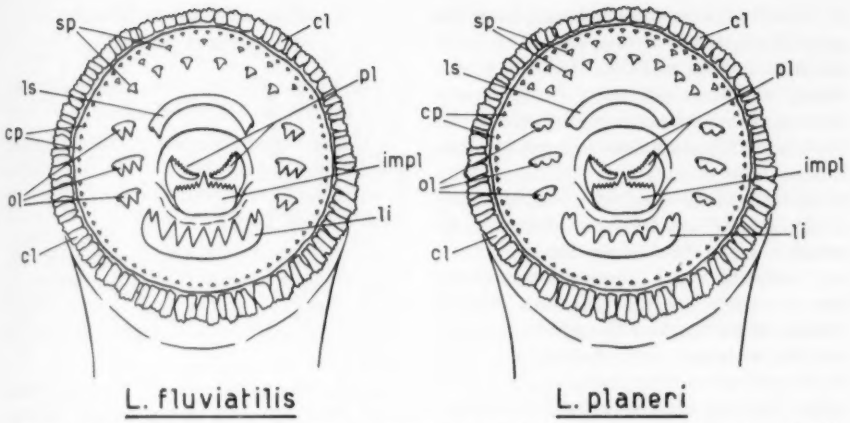


FIG. 2. Schematic drawing of the disc and dentition in the immature transformed specimens of the *Lampetra fluviatilis* and *L. planeri* (at sexual maturity they are very similar).
cl = oral fimbriae; *cp* = marginal series; *impl* = anterior lingual lamina; *li* = infraoral lamina; *ls* = supraoral lamina; *ol* = lateral teeth; *pl* = posterior lingual lamina; *sp* = rows of cusps on the anterior field.

Italy there are two allopatric species of brook lamprey. *L. zanandreai* lives only in the northern plain of Italy (usually called the plain of the Po), whereas *L. planeri* lives only in the Tyrrhenian and Ligurian tributaries.

Geographical distribution of lampreys in Italy.—From the problems of brook lampreys the author passed progressively to those of all lampreys in Italy. For this purpose he examined about 100 specimens of *Petromyzon marinus* caught at 57 localities and about 700 of *L. fluviatilis* collected at 26 localities. He also examined some specimens taken in neighboring lands bordering on the Mediterranean Sea (Jugoslavia, Monaco, France, and Spain). Recently he himself examined all Italian waters, including those of Sicily and Sardinia.

The first and very unexpected conclusion was that not a single *L. fluviatilis* was caught in the waters of the north Italian plain. All specimens from this region that had been attributed to this species proved to be small specimens of *P. marinus* or large examples of the brook lamprey *L. zanandreai*. The news greatly surprised both the author and the other Italian biologists. In this study *L. fluviatilis* was found only in the Tyrrhenian and Ligurian tributaries and in the basin of Rhône (France). The author has also caught this species in all streams tributary to the sea from Genoa to Salerno, but it is not present in Sicilian and Sardinian waters.

Thus, *L. fluviatilis* and *L. planeri* are sympatric and live in the same streams, at least in the Tiber, Garigliano, Volturno, Sarno, and Sele, from which some specimens of both species have been caught by the author himself. They can also be found together in the Rhône River in France. *Lampetra planeri* generally lives in the upper parts of the stream basins and *L. fluviatilis* in the lower parts, but in some places the two species occur in the same surroundings and have contemporary spawning seasons (Volturno and Sele). Recently, the habitat of *L. fluviatilis* has been reduced by dams that have been built along the river courses. In these places, of course, only *L. planeri* occurs above the dams.

L. zanandreai is allopatric in respect to both *L. fluviatilis* and *L. planeri*. No parasitic lamprey is morphologically similar to *L. zanandreai*, in the same sense that *L. planeri* corresponds with *L. fluviatilis* (fig. 2).

P. marinus may be found now, as in the past, in all Italian waters, and it has even run inland as far as Turin. It is caught also along the shores of Sicily, Sardinia, and Corsica, as well as along all Jugoslavian, French, and Spanish coasts of the Mediterranean. This species is sympatric with all other Italian lampreys, but it is caught very rarely, except in the lower Po, near its mouth close to the Adriatic Sea.

P. marinus and *L. fluviatilis* migrate to sea for their parasitic life. No notable damage to fish by these two species has been noticed, although some fishes have been found with wounds produced by these lampreys. In Italy there is no landlocked form of either species and the "praecox forma" of *L. fluviatilis* does not exist here, as it does in the Russian tributaries to the Baltic Sea (Berg, 1931, 1948).

Characters of Italian lampreys compared with those of the same or similar species of other countries.—The author has tried to examine thoroughly the biological, biometrical, and morphological characters of Italian lampreys, in order to compare them with the same and similar species of other countries.

His studies on the number of trunk myomeres, disposition and number of teeth both of oral disc and lingual laminae, and the coloration of the adults, have indicated that there is a perfect morphological correspondence between Italian specimens of *P. marinus* and those of other European and of North American waters. The same results were obtained in comparing Italian specimens of *L. fluviatilis* and of *L. planeri* with those of other European waters. (Cf. Berg, 1931, 1948; Ivanova-Berg, 1931, 1933; Vladykov, 1949; Applegate, 1950; Lanzing, 1959.)

L. zanandreai is similar in some morphological respects to the American *L. aepyptera*, in respect to the number of trunk myomeres, the number and disposition of teeth in the oral disc, and the coloration of the ammocoetes (cf. Seversmith, 1953, and Vladykov, 1955).

Conclusions From Studies of Lampreys in Italy

The following conclusions may be drawn from the studies discussed above:

(1) *Petromyzon marinus* in Italy reaches a maximum length of 870 mm, as it does in northern Europe, and as the anadromous form does in North America.

(2) *Lampetra fluviatilis* in Italy reaches a maximum length of 300 mm, whereas in north Europe it grows to 500 mm. There may, therefore, be a latitudinal cline in the adult size.

(3) *Lampetra planeri* and *L. zanandreai* reach about the same length (range, 85–200 mm), but in each species there are many differences in size between stocks from various localities (fig. 1), probably depending on nutrition and annual variation of water temperature.

(4) In all Italian lampreys ripe males and females differ in some body proportions, but the differences appear only at sexual maturity.

(5) The parasitic lampreys, as soon as their metamorphosis is completed, have larger eyes and longer heads than the nonparasitic ones.

(6) All Italian lampreys begin to metamorphose at the length of 100–200 mm, as do lampreys in other countries.

(7) *P. marinus* and *L. fluviatilis* complete their metamorphosis in fresh water and migrate to salt water. Even *L. fluviatilis* passes beyond brackish water.

(8) Sexual maturity in the migrating species is initiated in the sea and completed in fresh water.

(9) In Italy, these species migrate upstream between December and April, and spawn in the period from April to June. There is a single spawning run, not the two that are reported, for *L. fluviatilis*, in northern Europe.

(10) In Italy, *L. fluviatilis*, as well as *P. marinus*, preys in the sea on fish, as has been demonstrated by the author from a study of the food in the intestines.

The Danubian Lampreys

Early authors (Heckel und Kner, 1858; Wajgel, 1883; and Antipa, 1909) wrote that both *L. fluviatilis* and *L. planeri* occur in Danubian waters. Regan (1911) attributed the lampreys caught in the Rumanian tributaries of the Theiss River to a new genus and a new species, *Eudontomyzon danfordi*. Vladykov (1925–1931) came to the same conclusion for the lampreys of the Czechoslovakian tributaries of the Theiss. The lampreys taken in the lower basin of the Danube, as well as in all tributaries of the Black Sea, were attributed by Berg (1931) to another new species, *Lampetra mariae*, similar to *E. danfordi*.

Authors are not in agreement as to whether *E. danfordi* and *L. mariae* should be referred to Regan's genus *Eudontomyzon* (Vladykov, 1931; Chappuis, 1939; Oliva, 1953), or to a subgenus *Eudontomyzon* of *Lampetra* (Berg, 1931, 1948; Holly, 1933), or *Petromyzon* (Creaser and Hubbs, 1922). The writer refers *L. mariae* (Berg) to the genus *Eudontomyzon*.

The studies of the Danubian lampreys, as for those of Italy, were progressive. First, it was found that the Austrian lampreys are morphologically similar to *E. danfordi* and that they are nonparasitic; secondly, the same conclusions were drawn for the Zagabrian and Lubianian lampreys. At the same time *L. planeri* was found living in the Inn River (in the upper Danube basin) and in a small tributary of the upper Drava basin, near Klagenfurt, and *E. mariae* was found in a small tributary of the Sava River, near Sarajevo.

The examination of many specimens caught in the Theiss River, in both Rumanian and Czechoslovakian waters, shed much light on the status of *E. danfordi*, especially as some of these specimens had been examined by previous authors.

From these studies it is concluded that:

(1) *E. danfordi* is a parasitic lamprey (cf. Vladykov, 1926–1927), although it never migrates, not even in fresh water, from brook to river. Its morphological characters are those established by Regan and more accurately described by Vladykov (1931). According to the findings of Vladykov (1925–1931), or at any rate according to results of the author's study of specimens, this species lives in the tributaries of the Theiss in Rumania and Czechoslovakia.

(2) Most of the lampreys from Austria (from about Linz, Vienna, and Graz), some from Czechoslovakia (both in the Danube, near Bratislava, and in the Theiss, near Lukov), and all those of Zagabria and of Lubiana (in the Sava and Drava rivers), belong to a nonparasitic form, which the author (Zanandrea, 1959d) has named *E. danfordi vladykovi*, in honor of Prof. Vadim Vladykov (of Ottawa, Canada), an international lamprey specialist who has always been very kind in helping the writer. This form is morphologically similar to *E. danfordi*, in the same way that *L. planeri* is similar to *L. fluviatilis*. Both *E. d. danfordi* and *E. d. vladykovi* have very many teeth in all regions of the oral disc, and they have a continuous band of villiform teeth on the lower lip; on the contrary, *E. mariae* was indicated by Berg (1931), in describing the species, as having the number of teeth in the oral disc more or less reduced and the band of teeth in the lower lip noncontinuous. According to Berg, *E. mariae* varies much in respect to the number of teeth in the oral disc. He indicated that in some specimens they are almost as much reduced as in *L. planeri*, whereas in others the teeth are so numerous that they seem similar to those of *E. danfordi*. This variation needs more thorough study. In fact, a specimen from Bratislava that was incorrectly attributed to *L. planeri* has proved to be referable to *E. d. vladykovi* (Zanandrea and Oliva, 1959). The conclusion by Drensky (1951), that both *L. planeri* and *E. danfordi* occur in Bulgarian waters, also needs to be confirmed.

(3) Consequently, it can be concluded that in the Danube there is a new and clear case of paired forms of lampreys (see following). The same cannot be proved for *E. mariae*, although Berg (1931) implies this

possibility, on the basis of his interpretation of lampreys, caught in the Prut, that Wajgel (1883) regarded as parasitic. All specimens of *E. mariae* examined personally by the author, of which some are the same as those used by Berg in basing the species, belong to a nonparasitic lamprey, as do all specimens from Karkov recently studied by Balabai (1948, 1958).

Neoteny in a Lamprey

Neoteny, which has never been recorded in the lampreys, has now been observed by the writer. Among 200 ammocoetes and one adult male of *Lampetra zanandreai* collected in the small Fibbio Brook (in Verona, Italy) on March 18, 1955, twelve ammocoetes had well-developed ovaries containing eggs that were macroscopically visible on dissection and were in the third stage of development, as indicated by histological examination. These conclusions regarding the neotenic lampreys are based on the first results of the author's histological studies, made during his investigation of the normal development of the gonads in *L. zanandreai*.

One of these twelve larvae showed well-developed principal secondary sexual characters, namely, enlargement of the two dorsal fins, development of the anal pseudofin, and the transparent appearance of the body wall, through which the eggs could be seen. Such characters are normally associated only with adults that are about to spawn (cf. above). The neotenic specimens, all females, were collected during the spawning season.

Other neotenic ammocoetes, also females, were collected in November, 1956, in the Rosella, an irrigation brook, one kilometer below the derivation of the Rosella from the Fibbio. Here were taken a total of 221 ammocoetes 121–216 mm long and a female without the secondary sexual characters. The neotenic ammocoetes represent only about 20% of the series. Histological examination showed that the endostyle is intact and that the naso-pharyngeal pouch has the larval structure of the same species, as described by the author (1958c).

Experiments, designed to test the suspicion that this phenomenon is attributable to the effects of the effluent of a tannery two kilometers upstream from the localities in which the neotenic specimens were taken, did not give any conclusive results. In fact, many hundreds of marked ammocoetes caught near Treviso and Turin, and put into the Rosella and Fibbio brooks, metamorphosed and spawned normally. No conclusions, furthermore, were reached from a supplementary experiment, in which tadpoles of *Rana dalmatina*, a species that does not live there, were put into the same brooks during the first days of their development.

No neotenic lamprey was caught farther downstream in the same brooks, or in any other streams in which a great number of ammocoetes were taken.

These neotenic lampreys may be cited as examples of the attainment of sexual maturity in the absence of a definitive thyroid gland, which in lampreys, as is well known, is formed only during metamorphosis. This fact is very important because, although the endostyle has been shown to be capable of fixing iodine in an organic state, no signs of metamorphosis have been obtained by treatment with either anterior pituitary or thyroid-gland extract. All attempts to stimulate sexual maturity artificially in ammocoetes and in immature adults of various lampreys have produced discordant and questionable results. The principal attempts have been cited by the author (1956a).

Observations on the Speciation of Lampreys

As the studies on lampreys were continued many cases of speciation were encountered that appeared to be of interest to the biologist and to be of special value in the interpretation of the general problems of speciation.

The well-known landlocked forms, already mentioned above, do not need emphasis. This phenomenon is also exhibited by other fishes. It has been recorded for *P. marinus*, in which the landlocked form has, in the last 20 years, caused veritable calam-

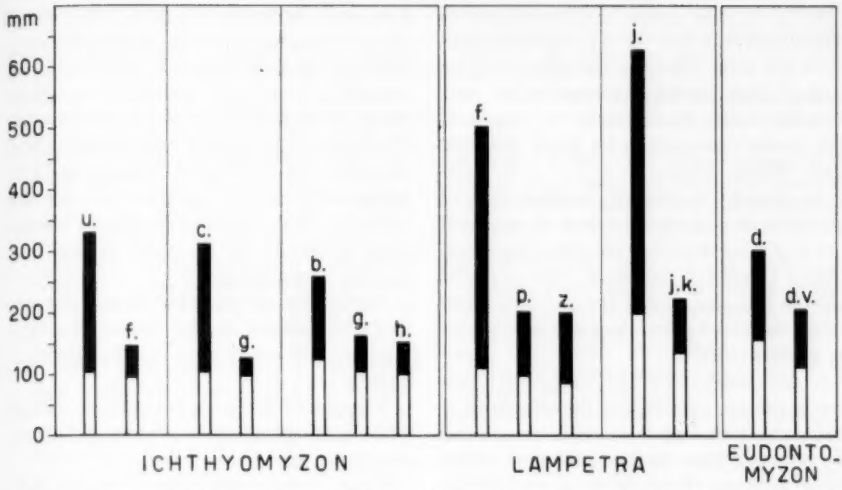


FIG. 3. Lampreys with paired forms (with range in length of metamorphosed specimens indicated by black bars).

u. = *Ichthyomyzon unicuspis*; f = *I. fossor*; c. = *I. castaneus*; g. = *I. gagei*; b. = *I. bdellium*; g. = *I. greeleyi*; h. = *I. hubbsi*; f. = *Lampetra fluviatilis* (in all Europe); p. = *L. planeri* (in all Europe); z = *L. zanandreai* (here included in order to compare it with other nonparasitic lampreys); j. = *L. j. japonica*; j.k. = *L. japonica kessleri*; d. = *Eudontomyzon d. danfordi*; d.v. = *E. danfordi vladykovi*.

ity for the commercial fisheries of the Great Lakes of North America (Applegate and Moffett, 1955). Not so well known is the landlocked form of *L. fluviatilis* of Lake Onega and Lake Ladoga in Russia (Berg, 1931).

The most significant speciational phenomenon exhibited by lampreys is the frequent development along parallel lines of two kinds that are morphologically very similar, but biologically very different, in that one feeds after metamorphosis, whereas the other does not. The first, or parasitic type, lives almost a year after metamorphosis in the adult stage; the second, a nonparasitic type, only a few months. Moreover, in the parasitic type the gonads begin to mature at end of the feeding stage, whereas in the nonparasitic type maturation proceeds during metamorphosis. This phenomenon was first pointed out as a generality by Hubbs (1925), and has been treated in more detail by Hubbs and Trautman (1937) and by the author (1959e), who has termed the related forms "paired" (fig. 3).

One case of paired forms, namely that of *Lampetra fluviatilis* and *L. planeri* (fig. 2), which was long the only case recognized, caused an infinite number of discussions among European authors. *L. planeri* was variously considered as a "forma minor," as a subspecies, or as an intermediate phase in the growth of *L. fluviatilis* (cf. Zanandrea, 1957b).

In the same genus *Lampetra*, according to authors who do not refer the species to *Entosphenus*, there exists a similar case, involving forms that have been called *L. j. japonica* and *L. j. kessleri*. Years ago they were attributed to *L. fluviatilis* and *L. planeri*, respectively. From the denomination of the nonparasitic form, it is clear that the Russian authors consider these forms as subspecies. The two forms live in northern Eurasian and northeastern Asiatic regions. The nonparasitic form alone occurs in an intermediate area of north Siberia, as do some other species of fish.

The most extended series of paired forms was discovered by Hubbs and Trautman

(1937), in the genus Ichthyomyzon of North America (cf. fig. 3). In this genus there are three parasitic lampreys, and for each of these there is a corresponding nonparasitic form. In one case, two nonparasitic forms correspond to a single parasitic form (Raney, 1952).

As already mentioned, another case of paired forms, *Eudontomyzon d. danfordi* and *E. d. vladykovi*, has recently been found among Danubian lampreys. The parasitic form in this pair, unlike the parasitic member of the other pairs, does not migrate for its parasitic life.

As previously remarked, the paired forms live in the same basin, but the nonparasitic form lives in the upper part of the basin and the parasitic form in the lower part of the basin, although there are cases in which the two forms occur in the same areas, and in the same spawning season.

There are many opinions about the origin of the paired forms. The more common view today is that each nonparasitic form has been derived from a parasitic species. For the genus *Ichthyomyzon*, Hubbs and Trautman (1937) believed that the parasitic *I. unicuspis* is the common ancestor, and that this species gave origin to the two other parasitic forms, *I. castaneus* and *I. bdellium*. Each of the three parasitic forms gave rise to a nonparasitic lamprey (*I. bdellium* to two such brook types, independently).

Many causes have been suggested regarding the origin of the nonparasitic forms from the parasitic. Young (1950) believed that the nonparasitic form is the product of paedomorphosis, and Leach (1951) attributed the precocious sexual maturity to the action of the anterior hypophysis. The findings on the neotenic lampreys seem to show a tendency in lampreys for sexual maturity to become accelerated.

One possible hypothesis can be offered to account for the phases through which the populations of parasitic lampreys, which run to the sea, become nonparasitic. A part of the parasitic population becomes landlocked and limits its run only to fresh water, as is now occurring in *Petromyzon marinus* and *Lampetra fluviatilis*. Then all lampreys of the species come to live in fresh waters only, as do the parasitic species of *Ichthyomyzon*, migrating from small streams to rivers or lakes. Still later they conserve the parasitic life, but do not migrate (*Eudontomyzon d. danfordi* is in this stage). Finally, in correlation with lack of prey, or because the action of the anterior hypophysis has become inhibited, or for other causes, they become nonparasitic.

Today the nonparasitic forms are generally considered to be species, but this question still needs to be investigated thoroughly.

The paired species must not be confused with "sibling" species, or with the abovementioned landlocked forms.

Other speciational phenomena in lampreys are interesting. One concerns *Petromyzon* and *Caspiomyzon*. Each of these genera has a single species, namely *P. marinus* and *C. wagneri*. The first is very well known; the second is indicated by Berg (1931) as having a surprising peculiarity: he indicated, without elaboration, that its intestine remains well developed and functional in the adult, but that it "does not carry a parasitic mode of life." It lives in the Caspian Sea tributaries.

Another case is quite the opposite. Some lamprey types exist over a considerable area only in the nonparasitic forms. This is the case of *L. zanandreai*, *L. aepyptera*, and *L. lamottenii* (also called *lamottei*, *appendix*, and *wilderi*, and commonly referred to *Eutosphenus*; cf. Trautman, 1957). *L. zanandreai* is limited to an area in Italy; the other two live in a rather large area in eastern North America. All three live in a region in which the ice was widely diffused during the Glacial Age, as Trautman emphasized for America, and as is well known for northern Italy. It cannot be decided if the ancestor of these species disappeared during the glaciations, or if these nonparasitic lampreys differentiated from other parasitic lampreys, still extant, of the same genus *Lampetra*.

Literature Cited

Antipa, Gregor. 1909. Fauna ictiologică a României. Public. Fond. Adamachi Acad. Română, **3** (16): 1–294.

Applegate, V. C. 1950. Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. U. S. Fish and Wildl. Serv., Spec. Sci. Rept.: Fish., **55**: 237 p.

—— and J. W. Moffett. 1955. The sea lampreys. Sci. Amer., **192** (4): 36–41.

Balabai, P. P. 1948. O vremeni i charaktere tecenija metamorfosa u *Lampetra mariae* Berg [The time needed in metamorphosis of *Lampetra mariae* Berg] (in Ukrainian). Trudi Inst. Zool. Akad. Nauk Ukrain. R. S. R., **1**: 29–38.

——. 1958. Metamorfos minoghi. Trudi Inst. Zool. Akad. Nauk Ukrain. R. S. R., **2**: 1–63.

Berg, L. S. 1931. A review of the lampreys of the northern hemisphere. Ann. Mus. Zool. Akad. Sci. U. R. S. S., **32**: 87–116.

——. 1948. Ryby presnych vod SSSR i sopredelnych stran. Faune SSSR . . . Zool. Inst. Akad. Nauk SSSR, **27** (1): 1–466.

Cotronei, Giulio. 1927. Ricerche morfo-ecologiche sulla biologia comparata dei Petromizonti. Parte 1. Pubbl. Staz. Zool. Napoli, **8**: 371–426.

Chappuis, P. A. 1939. Über die Lebensweise von *Eudontomyzon danfordi* Regan. Arch. Hydrobiol., **34**: 645–658.

Creaser, C. W., and C. L. Hubbs. 1922. A revision of the Holarctic lampreys. Occ. Pap. Mus. Zool. Univ. Michigan, **120**: 1–14.

Dendy, J. S., and D. C. Scott. 1953. Distribution, life history, and morphological variations of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia, **1953**: 152–162.

Drensky, P. 1951. Ribite y Bulgarija [Fishes of Bulgaria]. Fauna of Bulgaria No. 2, Zool. Inst. Bulgarian Acad. Sci., Sofia. 270 p.

Festa, E. 1900. Di un caso di icterismo nel *Petromyzon planeri* Bloch. Boll. Mus. Zool. Univ. Torino, 15 (367). 2 p.

Hardisty, M. W. 1944. The life history and growth of the brook lamprey (*Lampetra planeri*). J. Anim. Ecol., **13**: 110–122.

——. 1951. Duration of larval period in the brook lamprey (*Lampetra planeri*). Nature, **167**: 38–39.

Heckel, Jakob, und Rudolf Kner. 1858. Die Süsswasserfische der Österreichischen Monarchie, mit Rücksicht auf die angrenzenden Länder. Wilhelm Engelmann, Leipzig. 388 p.

Holly, Maximilian. 1933. Cyclostomata. *In:* Das Tierreich, Leipzig, Lief. **59**: 62 p.

Hubbs, C. L. 1925. The life-cycle and growth of lampreys. Pap. Michigan Acad. Sci., Arts and Letters, **4** (1924): 587–603.

—— and M. B. Trautman. 1937. A revision of the lamprey genus *Ichthyomyzon*. Misc. Publ. Mus. Zool. Univ. Michigan, **35**: 1–109.

Ivanova-Berg, M. M. 1931. Über die Lebensdauer der Larve von *Lampetra planeri* aus dem Gebiete des Finnischen Bunsen. Zool. Anz., **96**: 330–334.

——. 1933. Zur Biologie des Flussneunauges (*Lampetra fluviatilis* L.) Arch. Hydrobiol., **25**: 23–27.

Knowles, F. G. W. 1941. The duration of larval life in *Ammocoetes* and attempt to accelerate metamorphosis by injection of anterior-pituitary extract. Proc. Zool. Soc. London, (A) **111**: 101–109.

Lanzing, W. J. R. 1959. Studies on the river lamprey, *Lampetra fluviatilis*, during its anadromous migration. Doctoral dissertation. Vitgeversmaatschappij Neerlandie, Utrecht. 82 p.

Leach, W. James. 1951. The hypophysis of lampreys in relation to the nasal apparatus. J. Morph., **89**: 217–256.

Okkelberg, Peter. 1921. The early history of the germ cells in the brook lamprey, *Entosphenus wilderi* (Gage), up to and including the period of sex differentiation. J. Morph., **35**: 1–151.

——. 1922. Notes on the life-history of the brook lamprey, *Ichthyomyzon unicolor*. Occ. Pap. Mus. Zool. Univ. Michigan, **125**: 1–14.

Oliva, Ota. 1953. Příspěvek k přehledu našich mihulí (Petromyzones Berg 1940). [Contribution to the review of native lampreys (Petromyzones Berg 1940)]. Věstník Královské České společnosti nauk, Třída matematicko-přírodovědecká, **9**: 1–19. (In Czech, with English summary).

Raney, E. C. 1952. A new lamprey, *Ichthyomyzon hubbsi*, from the upper Tennessee River system. Copeia, **1952**: 93–99.

Regan, C. T. 1911. Synopsis of the marsipobranchs of the other *Hyperoartii*. Ann. Mag. Nat. Hist., (8) **7**: 193–204.

Schultz, L. P. 1930. The life history of *Lampetra planeri* Bloch, with a statistical analysis of the rate of growth of the larvae from western Washington. Occ. Pap. Mus. Zool. Univ. Michigan, **221**: 1–31.

Seversmith, H. F. 1953. Distribution, morphology and life history of *Lampetra aepyptera*, a brook lamprey, in Maryland. Copeia, **1953**: 225–232.

Trautman, M. B. 1957. The fishes of Ohio. Ohio State Univ. Press, Columbus, Ohio. 683 p.

Vladykov, Vadim. 1925. Über einige neue Fische aus des Tschechoslowakei (Karpathorussland). Zool. Anz., **64**: 248–252.

——. 1926. Ryby Podkarpatskoj Rusi i ich glavnejsie sposoby lovli. Užhorod. 147 p.

——. 1927. Zur Frage über das Absterben unserer Lampreten (Petromyzonidae) nach der Laichzeit. Zool. Anz., **74**: 237–239.

——. 1931. Poissons de la Russie Sous-Carpathique (Tchécoslavaquie). Mém. Soc. Zool. France, **29**: 217–374.

——. 1949. Quebec lampreys (Petromyzontidae). I: List of species and their economical importance. Dept. Fish. Quebec Contr. **26**: 1–67.

——. 1955. *Lampetra zanandreai*, a new species of lamprey from northern Italy. Copeia, **1955**: 215–223.

WAJGEL, LEOPOLD. 1883. Die Zusammenziehung der zwei Arten von *Petromyzon* (*P. planeri* und *P. fluviatilis*) in Eine. Verh. zool.-bot. Gesellsch. Wien, **33**: 311–320.

YOUNG, J. Z. 1950. The life of vertebrates. Oxford University Press, London. 767 p.

ZANANDREA, GIUSEPPE. 1940a. Curva peso-lunghezza e indice ponderale nell'accrescimento di *Petromyzon* (*Lampetra planeri* Bloch). Boll. Zool., **11**: 17–23.

——. 1940b. Osservazioni sulla diminuzione di lunghezza e di peso in relazione alla maturità sessuale nel *Petromyzon* (*Lampetra*) *planeri* Bloch. Arch. Zool. Ital., **29**: 77–87.

——. 1947. Notizie e appunti sui Petromizonti delle Tre Venezie. Boll. Pesca, Piscicolt. e Idrobiol., **23** (n. s., vol. 2): 125–128.

——. 1951. Rilievi e confronti biometrici e biologici sul *Petromyzon* (*Lampetra*) *planeri* Bloch nelle acque della Marca Trevigiana. Boll. Pesca, Piscicult. e Idrobiol., **27** (n. s., vol. 6): 53–78.

——. 1953. Note sulla ecologia e distribuzione in Italia della lampreda di ruscello (*Lampetra planeri* Bloch). Boll. Pesca, Piscicult. e Idrobiol., **29** (n. s., vol. 8): 252–269.

——. 1954. Corrispondenza tra forme parassite e non parassite nei generi *Ichthyomyzon* e *Lampetra* (problemi di speciazione). Boll. Zool., **21**: 461–466.

——. 1955a. La corrispondenza tra forme parassite e non parassite nei generi *Ichthyomyzon* e *Lampetra* nei confronti del problema della speciazione. Atti Acc. Sci. Bologna Rendic, (ser. 11) **2**: 1–16.

——. 1955b. Isolamento geografico della lampreda di ruscello nella pianura padana. Boll. Zool., **22**: 191–196.

——. 1956a. Neotenia in *Lampetra planeri zanandreai* (Vladykov) e l'endocrinologia sperimentale dei Ciclostomi. Boll. Zool., **23**: 412–427.

——. 1956b. Cenni sulla speciazione delle lamprede delle regioni oloartiche con particolare riferimento alle somiglianze tra *Lampetra aepyptera* e *Lampetra planeri zanandreai*. Boll. Zool., **23**: 429–439.

——. 1956c. Appunti sulle lamprede dell'Austria. Boll. Zool., **23**: 439–447.

——. 1956d. Le lamprede del Danubio: considerazioni e confronti. Boll. Pesca, Piscicult. e Idrobiol., **32** (n. s., vol. 11): 264–289.

——. 1956e. Sulla rigenerazione dei Petromizonti (*Lampetra planeri* Bloch). Atti Acc. Sci. Bologna Rendic., (ser. 11) **3**: 1–35.

——. 1957a. Neoteny in a lamprey. Nature, **179**: 925–926.

——. 1957b. Esame critico e comparativo delle lamprede catturate in Italia. Arch. Zool. Ital., **42**: 249–307.

——. 1958a. Posizione sistematica e distribuzione geografica di *Lampetra zanandreai* Vladykov. Mem. Mus. St. Nat. Verona, **6**: 207–237

——. 1958b. Le lamprede dei Musei di Zagabria e Lubiana. Bioloski Glasnik Zagreb, **11**: 45–62.

——. 1958c. Altri casi di lamprede neoteniche e il loro apparato naso-faringeo. Atti Istit. Veneto Sci. L. A., **116**: 179–194.

——. 1959a. Le lamprede della Campania. Pubbl. Staz. Zool. Napoli, **31**: 15–24.

——. 1959b. *Lampetra fluviatilis* catturata in mare nel Golfo di Gaeta. Pubbl. Staz. Zool. Napoli, **31**: 265–307.

——. 1959c. Speciation among lampreys. Nature, **184** (Suppl. 6): 380–381.

——. 1959d. Lamprede parassite e non parassite nel bacino del Danubio e la nuova entità sistematica *Eudontomyzon danfordi vladykovi*. Arch. Zool. Ital., **44**: 215–254.

——. 1959e. Recenti ricerche sulle forme "appaiate" di lamprede dell'Italia e del Danubio. Boll. Zool., **26**: 545–554.

—— E OTA OLIVA. 1959. Posizione sistematica di una lampreda di Cilistovo (Cecoslovacchia). Ann. Mus. Civ. St. Nat. Genova, **2**: (98): 1–5.

CHROMOSOMAL ADAPTIVE POLYMORPHISM IN *DROSOPHILA PERSIMILIS* III. MATING PROPENSITY OF HOMOKARYOTYPES[1]

ELIOT B. SPIESS AND BOZENA LANGER, *Department of Biological Sciences*
with appendix by C. C. LI, *Graduate School of Public Health*
University of Pittsburgh, Pittsburgh, Pennsylvania

Received April 10, 1961

The outcome of selection is determinate in laboratory populations of *Drosophila* containing variable proportions of gene arrangements of uniform geographic origin (Dobzhansky, 1954, 1957). Consequently such gene arrangements must carry genic complexes which confer adaptive functions upon their carriers. While the evidence for adaptive control by these complexes in populations of *D. pseudoobscura* and *D. persimilis*, for example, is overwhelming, the identification of those adaptive functions conferred by the genic complexes has remained somewhat cryptic. In the cases of chromosome III polymorphism in these species, "fitness," characters like viability, fertility, longevity, rates of development and maturation have not been proved to be intrinsic to the gene arrangement's adaptive control either in laboratory or natural populations, although many suggestive data have been accumulated (Spiess and Schuellein, 1956).

When raised under competitive conditions as in a population cage, flies with specific karyotypes may differ in combinations of adaptive traits which when considered separately are weak and barely measurable but which might bring about selective differences of considerable strength by their net action. Such combinations of fitness traits could account for a portion of changes observed in populations cages (Moos, 1954; Spiess, 1958). Logically it is to be expected that constant adaptive control of certain karyotypes in particular environments results from a physiological action which is capable of being ascertained in spite of genic interactions of some complexity and the sensitivity of such control to environmental variables. The net fitness of any gene arrangement karyotype may arise from the reinforcement of a considerable number of minor effects, or alternately it may arise from a single property inherent in the species' adaptation possibly with secondary functions reinforcing it.

This paper is the first report of a gene arrangement function in the *D. pseudoobscura* group which seems to be a function of sufficient strength and adaptive importance to the species to be worthy of further study.

In searching for fitness characters conferred by the Whitney (WT) and Klamath (KL) gene arrangements of chromosome III in *D. persimilis*, Spiess indicated in a previous paper of this series (1958) that homokaryotypes differed significantly in rate of pre-adult development but that they did not differ significantly in viability, fertility of females, or rate of maturation whether raised under crowded or near-optimal conditions. Heterokaryotypes were slightly heterotic for rate of development under near-optimal but intermediate under crowded conditions.

To test the adaptive strength of the development rate difference, populations were initiated in which food creamers were changed rapidly (= Fast Cages) to favor fast developing larvae (WT/WT) or changed slowly (= Slow Cages) to allow plenty of time for the other larvae (KL/KL and WT/KL) to emerge from food creamers before removal. The details of these experiments will be reported elsewhere, but it is sufficient to note that in the "fast cages" WT/WT was expected to be favored over WT/KL leading to elimination of the KL arrangement, while in the "slow cages" WT/KL and KL/KL should have been nearly equal to WT/WT. (The design of these experiments and rates of food creamer

[1] This work was carried out under Contract No. AT(30-1)-1775, U. S. Atomic Energy Commission.

TABLE 1. *Comparison between frequencies of introduced flies and the first egg sample in WT-KL population cages*

Cage #	Introduced adults			Egg sample (F_1 larvae)			
	WT/WT	KL/KL	No.	WT/WT	WT/KL	KL/KL	No.
FF	.204	.796	603	.14	.39	.47	100 Obs.
				.04	.33	.63	= Exp.
SS	.208	.792	616	.11	.53	.36	100 Obs.
				.04	.33	.63	= Exp.
FF-2	.251	.749	720	.25	.58	.17	100 Obs.
				.06	.38	.56	= Exp.
SS-2	.248	.752	722	.28	.48	.24	100 Obs.
				.06	.37	.57	= Exp.
DF	.244	.756	1016	.21	.48	.31	100 Obs.
				.06	.37	.57	= Exp.
DS	.250	.750	998	.03	.47	.50	100 Obs.
				.06	.38	.56	= Exp.
Average	.234	.766		.17	.49	.34	= Obs.
				.05	.36	.59	= Exp.

changes were based upon calculations given in Spiess, 1958.) Briefly, the outcome of selection in these cages did not fulfill these expectations: the KL arrangement decreased in both fast and slow cages at about the same rate, though final equilibrium was higher for KL by about 10% in the slow cages. Cages with discrete generations and overlapping generations were studied. Movement of larvae from creamer to creamer has been ruled out as a factor in nullifying the difference between these cages. It was therefore concluded that the WT arrangement must have been superior in other respects than in rate of development, and that the additional superiority was strong enough to overcome the rate of development difference.

A clue to the more significant adaptive function appeared in a discrepancy between proportions of arrangements among flies introduced into the population cage as compared with the sample count of arrangements (control count) among F_1 larvae (see table 1). Flies used for initiating cages were homokaryotypes only (hybrids from strain crosses), and had been matured with sexes isolated for 10 to 20 days at 15° C. The WT arrangement increased consistently in every cage between introduction of the flies and egg-laying apparently: the zygotic frequencies of F_1 larvae showed a consistently large deficiency for KL/KL and excess of WT/KL. Differential viability of larvae in sample bottles before salivary analysis and greater fecundity of WT/WT females were ruled out as contributory to this discrepancy. Faster rate of development by WT larvae was a possible factor except that in salivary analysis this error is minimized by examining chromosomes from the midpoint of average developmental time. A mating propensity difference in one or both sexes was therefore suspected to exist between WT/WT and KL/KL. It is very important to notice that the average zygote frequencies for these six population cages agrees with a fit to the square of their gamete frequencies. This apparent "Hardy-Weinberg fit" can have come about only if chromosome frequencies were effectively equal in the two sexes. An equal boost in WT/WT male and female frequencies by a factor of 2.4, for example, will produce a nearly perfect fit to these average zygote data (see Appendix by C. C. Li). Obviously an increase of that sort might have come about by an increase in mating propensity of both sexes simultaneously. An increase in one sex only, while it might account for an increase in total amount of WT, cannot account for the fit to the square of the chromosomal frequencies.

MATERIALS AND METHODS

To account for the discrepancy in chromosomal frequency between introduced adult flies and their F_1 egg sample, three experiments were devised:

Experiment 1. To measure the ability of each karyotype male to inseminate a number of females a single male together with ten females of uniform karyotype were mated for 24 hours in a half-pint bottle provided with yeasted food in a plastic spoon. With four strains of WT and four of KL from White Wolf locality (8,000 ft elevation), California, four series of crosses were made to provide homokaryotype F_1 flies (WT/WT or KL/KL) for testing. The

same crosses were made as those described in Spiess and Schuellein (1956), that is, crossing four strains of WT (1 × 2, 2 × 3, 3 × 4, and 4 × 1) provided the F_1 series A–D, while the F_1 series of KL crosses provided E–H. A diallele set of all eight series males by eight series females served to randomize the matings. Within each of the 64 matings three replicates were run, giving a total of 192 males tested to 1,920 females.

Sexes were isolated at emergence from the pupa and stored for 10–15 days, then flies were mated without etherization and immediately placed in constant temperature (15° C). At the end of 24 hours females were dissected in isotonic Hoyle's solution, and their reproductive tracts were examined for live sperm. No attempt was made to measure volume of sperm present. If any live sperm were observed in either spermathecae, ventral receptacle, or vagina, the mating was positive.

Experiment 2. To measure relative mating propensity among rival males and receptivity among different females such as certainly occurs at the initiation of a population cage, five males and 10 females of each karyotype were mated (30 total flies), for 24 hours in half-pint bottles as before. To economize the number of possible combinations only the following four combinations of WT and KL were mated: A and E, B and H, C and G, D and F (A–D = WT, E–H = KL F_1 from strain crosses as before). Three replicate matings were run for each combination, so that a total of 120 males were tested to 240 females.

Identification of flies mating in these mixed cultures was accomplished as follows: females were colored with a dust mixture of dye powder plus wheat flour (method of Spieth, 1955). The dyes, neutral red and methylene blue, were used alternately on WT or KL females in successive matings. Twenty-four hours were given to the females to clean off the dust mixture from their bodies before mating. Enough dye particles remain in corners of the body which the fly cannot reach for identification even after two weeks. After mating the flies for 24 hours, females were isolated in separate vials, one female per vial. A single F_1 larva's salivary gland complement was sufficient to identify the male parent.

TABLE 2. *Experiment 1: single ♂ × 10 ♀♀. The average number of ♀♀ inseminated in 24 hours by a single ♂. Each average ($\bar{X}$) = propensity of 48 ♂♂, with 3 ♂♂ in each of 16 different series matings*

	WT ♀♀	KL ♀♀	Av. for ♂♂
WT ♂	$\bar{X} = 6.58$	$\bar{X} = 6.21$	$6.40 = 1.36$
KL ♂	$\bar{X} = 5.15$	$\bar{X} = 4.25$	$4.70 = 1.00$
AV.	5.86	5.23	
for ♀♀	$= 1.12$	$= 1.00$	

Experiment 3. The methods were identical with those of experiment 2 except that equal numbers of the sexes were mated (10 males with 10 females of each karyotype per bottle). With 40 flies per bottle and three replications for each of four combinations, a total of 240 males were tested with 240 females.

RESULTS

Experiment 1. The average number of females inseminated in a 24-hour period by a single male is given in table 2. These data express the total capability for any single male of a given homokaryotype in terms of both mating propensity, or "drive," and sperm volume available. Sperm volume was not measured, so these data by themselves do not discriminate between the hypotheses that males may have about equal sperm volumes but different mating propensity and the converse, that sperm volumes differed but not mating propensities. Nevertheless, individual male frequencies can be used for comparison with the experiments to follow.

The data indicate that WT males mate more than one-third again as frequently as KL males. In table 3 the analysis of variance demonstrates that this difference is very highly significant (the *F* value for WT males vs. KL males is more than four times greater than the 1% probability *F* value). WT females average slightly higher than KL females in receptivity to both kinds of

TABLE 3. *Experiment 1: Analysis of variance*

Variation	Sum Sq's	d.f.	Mean Sq.
Total	1310.74	63	
Between karyotype matings	483.05	3	
Orthogonal contrasts			
1) WT ♀♀ vs. KL ♀♀	58.14	1	58.14
2) WT ♂♂ vs. KL ♂♂	415.14	1	415.14
3) Homogamic vs. heterogamic	9.77	1	9.77
Subtotal	483.05	3	
Within karyotype matings	827.69	60	13.79

1) F (WT ♀♀ vs. KL ♀♀) = 4.22
2) F (WT ♂♂ vs. KL ♂♂) = 30.10
3) F (homog. vs. heterog.) < error
F @ 5% = 4.00
@ 1% = 7.08

males. However, the difference between females is only slightly significant (the F value of 4.22 having a probability of about 4.8%). When detailed data are examined (see table 4), it is evident that this difference is brought about exclusively by one high WT series (A) and one very low KL series (G). The other three series of WT (B, C, D) and KL (E, F, H) average 5.5 females and 5.6 females inseminated respectively; consequently the difference between WT and KL females vanishes when these exceptional series are omitted.

Finally, there is no evidence for preferential mating with an insignificant difference between homogamic and heterogamic matings (F value is less than error).

There is an interesting correlation between the sexes in each series of crosses. In table 4 is given the number of inseminations according to each series cross. For the WT males and their corresponding sisters there is a significant negative correlation ($r = -0.96$), that is, males which accomplish fewer inseminations have sisters which are inseminated more often. Possibly this effect implies that genotypes which produce less sexual activity in males correspondingly produce in females either weakness in repelling males or greater receptivity (which may be the case is impossible to say without examining the behavior of these genotypes in detail). This relationship holds only partially and not significantly for the KL series of crosses ($r = -0.36$).

TABLE 4. *Mating frequency of ♂♂ and number of ♀♀ inseminated for each series cross (experiment 1)*

Arrangement	Series	♂♂	♀♀
WT	A	5.92	7.08
	B	6.46	5.79
	C	6.42	5.46
	D	6.79	5.13
KL	E	4.13	5.33
	F	4.42	5.92
	G	5.00	4.17
	H	5.25	5.50

Experiment 2. When several individuals of each sex and gene arrangement (multiple choice) are mated, any activity differences between WT and KL flies should be intensified and at the same time the relative importance of sperm volume as a limiting factor in mating frequency should be minimized. That is to say, if the higher frequency of WT mating was simply due to greater sperm volume in those males, and consequently to their depositing of sperm among more females rather than due to a greater "drive" to mate, then under a state of rivalry the greater sperm volume would be expected to be ineffectual as long as the "drives" of WT and KL males were equal.

Table 5 summarizes the data obtained

TABLE 5. *Experiment 2: Number of matings in twenty-four hours using 240 ♀♀ and 120 ♂♂ randomized from four series of strain crosses, equal numbers from each series. Each mating bottle with 5 WT ♂♂, 5 KL ♂♂, 10 WT ♀♀, and 10 KL ♀♀ and run in triplicate*

	WT ♀♀			KL ♀♀			Total for ♂♂
	Series		Total	Series		Total	
WT ♂♂	A–E	19	85	A–E	19	56	141
	B–H	23		B–H	17		
	C–G	22		C–G	8		= 2.51
	D–F	21		D–F	12		
KL ♂♂	A–E	10	30	A–E	11	26	56
	B–H	6		B–H	9		
	C–G	8		C–G	2		= 1.00
	D–F	6		D–F	4		
Total for ♀♀			115			82	
			= 1.40			= 1.00	

TABLE 6. *Experiment 2: Analysis of variance*

Variation	Sum Sq's	d.f.	Mean Sq.
Total	705.44	15	
Between karyotype matings	558.68	3	
Orthogonal contrasts			
1) WT ♀♀ vs. KL ♀♀	68.06	1	68.06
2) WT ♂♂ vs. KL ♂♂	451.56	1	451.56
3) Homogamic vs. heterogamic	39.06	1	39.06
Subtotal	558.68	3	
Within karyotype matings	146.76	12	12.23

1) F (WT ♀♀ vs. KL ♀♀) = 5.57
2) F (WT ♂♂ vs. KL ♂♂) = 36.92
3) F (homog. vs. heterog.) = 3.19
F @ 5% = 4.75
@ 1% = 9.33

when five males and 10 females of each arrangement occupy the same mating bottle. It is quite evident that WT males mate much more frequently than KL males under these conditions of rivalry, a fact which would favor "drive" rather than greater sperm volume as the major factor in producing this difference. For every KL male which mated, 2.51 WT males mated. This ratio is very highly significant as can be seen from the analysis of variance in table 6. It is identical with the ratio utilized by Petit (1958), known as the "coefficient of sexual selection" which she has found useful in describing mating propensity differences in competition experiments with *D. melanogaster*:

$$K = \frac{A/a}{B/b},$$

where A = number of females inseminated by WT males
a = number of WT males in the population
B = number of females inseminated by KL males
b = number of KL males in the population

The standard error for this coefficient calculated by L'Heritier for Petit can be used here for comparisons between experiments: $SE_K = \sqrt{b^2/a^2 \cdot N\, A/B^3} = 0.439$ for the data in experiment 2.

Originally this experiment was designed to measure female activity before it was known how many times a single male might mate in 24 hours. If a male mated once only, and if 10 WT females paired off with all 10 males leaving no KL females mated, the WT females could have been designated 100% active (or receptive) and the KL 0%. Since males did not perform so simply, most females in each bottle were inseminated; WT males on the average must have mated twice and 21 had to mate three times to accomplish 141 inseminations, while KL males mated once on the average with four not mating; however, only five WT females failed to be inseminated (or were sterile), while 38 KL females were non-productive. It can be seen in table 5 that there were a few exceptions to the higher productivity of WT females: in the A–E bottles and in B–H (by KL males), KL females were inseminated either equally or more frequently than WT. In table 6 the analysis of variance F ratio for the female difference is slightly significant with a probability about 3%; it seems reasonable then that there is a general slight superiority of WT females under these conditions. Of course with WT males succeeding in mating more frequently also, the WT arrangement has a very marked advantage.

Experiment 3. Table 7 summarizes the

TABLE 7. *Experiment 3: Number of matings in twenty-four hours, using 240 ♀♀ and 240 ♂♂, randomized from four series of strain crosses, equal numbers from each series. Each mating bottle with 10 WT ♂♂, 10 KL ♂♂, 10 WT ♀♀, and 10 KL ♀♀ and run in triplicate*

	WT ♀♀			KL ♀♀			Total for ♂♂
	Series		Total	Series		Total	
WT ♂♂	A–E	19	75	A–E	13	65	140 = 2.19
	B–H	16		B–H	19		
	C–G	17		C–G	9		
	D–F	23		D–F	24		
KL ♂♂	A–E	9	32	A–E	15	32	64 = 1.00
	B–H	14		B–H	10		
	C–G	5		C–G	5		
	D–F	4		D–F	2		
Total for ♀♀			107 = 1.10			97 = 1.00	

TABLE 8. *Experiment 3: Analysis of variance*

Variation	Sum Sq's	d.f.	Mean Sq.
Total	701.44	15	
Between karyotype matings	383.18	3	
Orthogonal contrasts			
1) WT ♀♀ vs. KL ♀♀	5.06	1	5.06
2) WT ♂♂ vs. KL ♂♂	370.56	1	370.56
3) Homogamic vs. heterogamic	7.56	1	7.56
Subtotal	383.18	3	
Within karyotype matings	318.26	12	26.52

1) F (WT ♀♀ vs. KL ♀♀) $<$ error
2) F (WT ♂♂ vs. KL ♂♂) $= 13.97$
3) F (homog. vs. heterog.) $<$ error
F @ 1% $= 9.33$

TABLE 9. *Estimation of the degree of mating propensity (based on data of table 1)*

Cage	$u = p/q$	$u' = p'/q'$	$k = u'/u$	$V(k)$	$w = 1/V(k)$
FF	.2563	.5038	1.97	.0867	11.53
SS	.2626	.6000	2.28	.1113	8.98
FF-2	.3351	1.1739	3.50	.2470	4.05
SS-2	.3298	1.0833	3.28	.2162	4.63
DF	.3228	.8182	2.53	.1298	7.70
DS	.3333	.3605	1.08	.0300	33.30
6-cage average		$\Sigma wk = 128.00$		$\Sigma w = 70.19$	
		$\bar{k} = 1.82$		$V(\bar{k}) = 0.014$	
4-cage average (excluding FF-2 and DS)		$\Sigma wk = 77.86$		$\Sigma w = 32.84$	
		$\bar{k} = 2.37$		$V(\bar{k}) = 0.030$	

mating frequencies when the sexes are equal in numbers, 10 males and 10 females of each gene arrangement per mating bottle. Again WT males mate more than twice as often as KL males. Using the Petit coefficient, $K = 2.19 \pm 0.358$, it is not significantly different from the index in experiment 2. It should also be noted that, while the indices are about the same for the two experiments, in this third experiment every WT male mated once on the average with 20 mating twice, while of the KL males only about half were successful. Under greater competition all the WT males can be considered capable of transmitting their heredity but only half the KL males can be so considered.

WT females are again inseminated slightly more often but not significantly more than KL females. In table 8 the analysis of variance shows a significant difference only between males. If there was a real difference between WT and KL female inseminations when half as many males were present, such a difference no longer exists when an abundance of males is available: if females were discriminating or different in receptivity before (or if all males preferred WT females to KL before, which is less likely), the addition of males either erases the female choice or male preference or both.

Ironically, the total number of non-productive females is not much less: 36 (13 WT and 23 KL) non-productive females in this experiment compared with 43 (5 WT and 38 KL) when half as many males are present. These data indicate that increasing the total males changes the mating propensity differences of females, but there is a nearly constant refractory fraction of non-productive females. If females were non-productive, vials were examined for eggs. Unhatched eggs were recorded as evidence of virginity, while lack of eggs after three weeks indicated female sterility. The latter was apparently constant in both experiments 2 and 3 (22 and 26 females respectively produced no eggs; but the number of virgin females was twice as great in experiment 2 as in 3 (21 and 10 females respectively). The irony of doubling the number of males without decreasing non-productivity of females becomes less troublesome then. The constancy of female sterility at about 10% in both experiments implies the constant segregation of sterility genes in these crosses.

DISCUSSION

Success of mating depends predominantly upon the males activity, or "drive," in finding a female and his ability to proceed with a series of stimuli which elicit appropriate responses in the female to which he may respond eventually by copulation, and secondly upon the female's receptivity, or reactivity, to the male's advances by eliciting

those responses which encourage him to proceed (Bateman, 1948; Spieth, 1952; Bastock, 1956). In addition to these behavioral facts the amount of sperm which the male has available and the amount he transfers at each copulation may limit the number of females he can inseminate in a given time interval; however, mating activity and sperm volume may be quite independent. According to J. Maynard Smith (cited by Bastock, 1956) "mutant males of *D. subobscura* which lack a testis are just as successful in mating as wild ones." At least it is the number of females inseminated rather than an excess of sperm delivered at a single insemination which is probably more critical to a population in which time for mating and survival is limited, assuming that enough sperm are delivered on the average sufficient to provide for egg fertility (Bateman, 1948). It is well known that once a female mates she is less likely to mate again for at least one hour (Mayr, 1946), so that if two types of male compete simultaneously for mating with a given group of females that type of male which mates with more females in a limited time will contribute more progeny than the type male that may mate less frequently but contribute more sperm at each insemination.

Without measuring sperm volumes it might be difficult to prove by the first experiment (single male × 10 females) whether the frequency of inseminations is simply a function of total sperm volume or not. The increase in WT male mating frequency relative to KL when both kinds of male are participating certainly nullifies the sperm volume hypothesis, however. It is far more likely that a behavioral difference exists which brings about more extensive mating ability on the part of WT males. This difference in male behavior is not quite sufficient to explain the discrepancy between proportions of gene arrangements among flies introduced into population cages and the proportions of those arrangements in the first egg sample (table 1): the average increment observed in those cage data is 0.180 WT (from 0.234 to 0.414 between adult parents and the F_1 egg sample). If the coefficient for males in experiment 2 ($K = 2.51$) is used to calculate the frequency of WT among the F_1 eggs, the increment expected amounts to 0.102. If the additional coefficient for females (1.40) is also used, the increment in WT comes to 0.134. The population cages concerned were initiated with equal numbers of males and females, however, so the data from experiment 3 should apply. With no evidence for female differences in experiment 3, the increments of greater than 10% seem to require more than the advantage observed for males in these current experiments.

There remains a possibility that the coefficient of mating propensity may be much higher when WT males and females are much less frequent than KL. Petit (1958) has demonstrated a very interesting feature of the mating propensity in *D. melanogaster* which may be quite pertinent to this work: she has found by varying the male relative frequencies of certain sex-linked mutants (Bar and white eye vs. their wild-type alleles respectively) that the coefficient of sexual selection may change considerably as the mutant is increased in frequency with respect to its allele. For example, the white eye males are more efficient than their wild-type competitors in mating when the frequency of white males is less than 40% or higher than 80%. These facts have led her to propose a variation in the coefficient of sexual selection as a principal cause in bringing about some observed changes in relative adaptive values of laboratory populations (as in Spiess, 1957). Much work needs to be done in the extension of these experiments by varying the relative proportions of WT and KL. When WT is low in frequency, its coefficient of sexual selection may be much higher than that measured in these experiments, for example.

The male propensity is obvious, but the probability that female receptivity might change also is evidenced by the outcome of experiment 2. Not only might it change

with low frequency of WT, but there might be an optimal sex ratio for expression of WT female behavior.

Though the observed increment in WT is not completely accountable by the mating propensity values obtained when using equal numbers of WT and KL males, the effect is nonetheless of obvious importance when it is consistently significant in all three experiments. It can hardly be inconsequential to the total adaptive values of the genic complexes included within the WT and KL gene arrangements. As far as we are aware, this is the first demonstration of a behavioral property clearly controlled by these naturally occurring chromosomal variants.

Since sexual behavior is so important in the origin of isolating mechanisms between species of *Drosophila*, the genetic elucidation of mating behavior at subspecific and population levels becomes indispensable. The work of Bastock (1956) and Petit (1958) on behavior of single mutant loci in *D. melanogaster* demonstrates that selection acts on the courtship pattern to "gear" the sexes for highest efficiency of mating. The mechanism of chromosomal polymorphism in *Drosophila* may yet be shown to have a very crucial role to play in the evolution of mating behavior within populations.

Summary

In a search for fitness characters conferred by the Whitney and Klamath gene arrangements of chromosome III in *D. persimilis*, rate of pre-adult development had been ascertained as significantly controlled by these arrangements; but when tested by population cage technique differences in development did not effect expected differences in relative frequency changes of those arrangements. Consequently a more potent fitness character was looked for. A discrepancy between gene arrangement proportions among adult flies and their egg samples at the initiation of the population cages indicated a possible mating propensity difference between flies carrying these arrangements.

Three experiments were designed to test the mating propensity differences between homokaryotypes (WT/WT or KL/KL) of both sexes: (1) The average number of females inseminated in 24 hours by a single male, (2) the number of matings when five males and 10 females of each arrangement (30 total) occupy in the same mating bottle, and (3) the number of matings when 10 of each sex and each arrangement (40 total) occupy in the same bottle. All flies tested were F_1's of strain crosses. Matings were done at 15° C in randomized sets.

In all three experiments WT males mated significantly more often than KL males. This difference is less when single males are mated with 10 females (experiment 1: ratio of 1.36 for WT males to 1.00 for KL males), and greater in the multiple-choice experiments 2 and 3 (average ratio of 2.35 for WT males to 1.00 for KL males). Sperm volume was not measured, but in view of the fact that conditions were competitive in the multiple-choice experiments, mating behavior rather than sperm volume differences must account for the frequency of mating.

WT and KL females showed no uniform and significant differences in mating except in experiment 2 (with a sex-ratio of 2:1), where WT females mated more frequently than KL females by a ratio of 1.4 to 1.0. Female sterility was constant at about 10% for the two multiple-choice experiments as determined by lack of egg-laying. No significant preferential mating was observed.

The observed difference in male mating propensity is not quite sufficient to account for the average discrepancy between proportions of gene arrangements among adult flies and egg samples in population cages. There remains the possibility that the coefficient of mating propensity may be much higher when the gene arrangements are not present in equal frequency.

The adaptive role of chromosomal polymorphism in *Drosophila* may well have a behavioral basis, a possibility which seems never to have been investigated heretofore. In view of the prominence of mating behavior in isolating mechanisms between species

of *Drosophila* and the likelihood of its adaptive value to a population, it is essential to extend the knowledge of intrapopulational variation in mating behavior.

Acknowledgments

The authors are very grateful to Dr. Lee Ehrman, Professors T. Dobzhansky, and C. C. Li for reading the manuscript and for their valuable suggestions and criticisms.

Literature Cited

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity, **2**: 349–368.

Bastock, M. 1956. A gene mutation which changes a behavior pattern. Evolution, **10**: 421–439.

Dobzhansky, T. 1954. Evolution as a creative process. Caryologia, **6**: 435–449.

——. 1957. Mendelian populations as genetic systems. Cold Spring Harbor Symposium Quant. Biol., **22**: 385–393.

Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *D. pseudoobscura* and *D. persimilis*. Proc. Nat. Acad. Sci., **32**: 128–137.

Moos, J. R. 1955. Comparative physiology of some chromosomal types in *D. pseudoobscura*. Evolution, **9**: 141–151.

Petit, C. 1958. Le déterminisme génétique et psycho-physiologique de la compétition sexuelle chez *D. melanogaster*. Bull. Biol. France et Belgique, **92**: 248–329.

Spiess, E. B. 1957. Relation between frequencies and adaptive values of chromosomal arrangements in *D. persimilis*. Evolution, **11**: 84–93.

——. 1958. Chromosomal adaptive polymorphism in *D. persimilis*. II. Effects of population cage conditions on life cycle components. Evolution, **12**: 234–245.

—— and Schuellein, R. J. 1956. Chromosomal adaptive polymorphism in *D. persimilis*. I. Life cycle components under near optimal conditions. Genetics, **41**: 501–516.

Spieth, H. T. 1952. Mating behavior within the genus *Drosophila* (Diptera). Bull. Amer. Museum Nat. Hist., **99**: 399–474.

——. 1955. Aniline marking dye for live *Drosophila*. Drosophila Info. Service, No. **29**: 178.

Appendix

Estimating the degree of mating propensity.

C. C. Li

The results of mating experiments reported by Spiess and Langer suggest that flies of the genotype WT/WT have a higher mating propensity than KL/KL flies under the specified conditions. The observed numbers of three genotypes in F_1 larvae (table 1) are reasonably in agreement with Hardy-Weinberg proportions. This fact suggests that the mating propensity in males and females is of the same degree or very approximately so. The following method of estimating the degree of mating propensity assumes that it is the same in the two sexes.

Let p and q denote the proportion of WT/WT and KL/KL parent flies initially introduced into the mating cage, and let $k : 1$ be the relative mating propensity of the two parental genotypes (WT/WT : KL/KL). Then the expected proportions of the three genotypes in F_1 larvae are the terms of $(kp + q)^2$, viz.,

WT/WT	WT/KL	KL/KL	Total
k^2p^2	$2kpq$	q^2	$(kp + q)^2$

acounting for the observed fact that F_1 larva numbers conform with binomial proportions. The frequencies of WT and KL chromosomes in the F_1 generation are:

$$p' = \frac{k^2p^2 + kpq}{(kp + q)^2} = \frac{kp}{kp + q},$$

$$q' = \frac{kpq + q^2}{(kp + q)^2} = \frac{q}{kp + q}$$

Since the initial parental frequencies (p, q) are fixed by the investigators and the subsequent frequencies in the F_1 sample (p', q') are observed, an estimate of k may be obtained by solving either of the two equivalent equations shown above. Thus,

$$k = \frac{p'q}{q'p} = \frac{p'/q'}{p/q} = \frac{u'}{u}$$

where $u = p/q$ and $u' = p'/q'$ are the frequency ratios.

The variance of the estimate k is easily found, because u is a fixed value and has no sampling variation. Hence, $V(k) = V(u')/u^2$. If n is the sample size of F_1 larvae, it is known that $V(u') = u'(1 + u')^2/$

$2n$ (see Li, 1955. *Population Genetics*, p. 24). Hence,

$$V(k) = \frac{u'(1+u')^2}{2\,n\,u^2},$$

the square root of which is the standard error of k.

Example: In the first cage (FF of table 1), the initial parental frequency ratio is $u = 204/796 = 0.25628$, and the subsequent F_1 frequency ratio is $u' = (28+39)/(39+94) = 67/133 = 0.50376$. Hence, $k = u'/u = 1.966$. The sample size is $n = 100$, yielding

$$V(k) = \frac{0.50376(1.50376)^2}{200(0.25628)^2} = 0.0867;$$

$$\text{S.E.}(k) = 0.294.$$

Proceeding the same way with the other cages, we obtain table 9, in which the values of k have been rounded to two decimal places.

It is to be noted from table 1 that the observed numbers in F_1 larvae from cages FF-2 and DS are not quite in accord with Hardy-Weinberg proportions, and one yields the highest estimate of mating propensity and the other the lowest. The results of these two cages are apparently the least reliable. It is thought that either there has been extraordinary sampling variation in these two cases, or the model proposed here does not approximate the true biological situation. The mating propensity could well be different for males and females. The data as presented in table 1 do not permit separate estimates of propensity in the two sexes. It is also conceivable that mating propensity may be easily influenced by other genetical and environmental factors.

Average estimate. The first two cages (FF and SS) have very nearly the same initial chromosome frequencies. The average value of p may be taken as $\frac{1}{2}(0.204 + 0.208) = 0.206$, so that $u = 206/794$. The observed numbers in these two F_1 samples may be pooled to give 25, 92, 83 (table 1) so that $u' = 71/129 = 0.5504$. The average estimate and its variance for these two samples of total size 200 are then

(FF and SS), $\bar{k} = 2.12$

$V(\bar{k}) = 0.0491$.

This pooling method is applicable when the cages have the same initial chromosome frequencies.

A more general method is to take the weighted average value of the separate k's, the weight being the reciprocal of $V(k)$ shown in the last column of table 9. Thus, for the first two F_1 samples (FF and SS), we have

$$\bar{k} = \frac{\Sigma\, w\, k}{\Sigma\, w} = \frac{11.53(1.97) + 8.98(2.28)}{11.53 + 8.98}$$

$$= \frac{43.19}{20.51} = 2.11$$

$$V(\bar{k}) = \frac{1}{\Sigma\, w} = \frac{1}{20.51} = 0.0488.$$

It is seen that the weighting method yields practically the same results as the pooling method, because the initial chromosome frequencies are almost the same in these two cages. If this is not the case, the weighting method is preferable.

The average k based on all six cages is found to be 1.82 ± 0.12 by the weighting method (see bottom of table 9). Eliminating the two cages (FF-2 and DS) which are not quite in agreement with Hardy-Weinberg law, we find that the average k for the remaining four cages is 2.37 ± 0.17. For many purposes, a sample of 100 observations must be considered fairly adequate. In studying mating propensity, however, it is thought that samples of a larger size would elucidate the situation much more clearly. On the face value of the observed results we would say that the degree of mating propensity, WT/WT vs. KL/KL, is probably between 2.00 and 2.70.

THE EAST PACIFIC BARRIER AND THE DISTRIBUTION OF MARINE SHORE FISHES

JOHN C. BRIGGS[1]

Institute of Fisheries, University of British Columbia, Vancouver, British Columbia, Canada

Received April 28, 1961

Although the East Pacific Barrier has been the object of considerable speculation by contemporary marine zoologists, it was Charles Darwin (1872: 131) who first recognized its zoogeographic importance. "Westward of the shores of America, a wide space of open ocean extends, with not an island as a halting-place for emigrants; here we have a barrier of another kind, and as soon as this is passed we meet in the eastern islands of the Pacific with another and totally distinct fauna."

As Ekman has emphasized (1953: 73), the East Pacific Barrier is considered by many to be responsible for the most pronounced break in the circumtropical shore fauna. It has been noted recently (Briggs, 1960) that 14 species of circumtropical shore fishes have succeeded in crossing this area. The number should now be reduced to 13, for Garrick (1960) has shown that the bramble-shark genus *Echinorhinus* is not monotypic and that, so far, neither of the two species can be considered cosmopolitan. As nearly as can be determined from the various checklists, faunal works, and recent revisionary literature as well as an examination of specimens of certain species, in addition to the 13 worldwide fishes, 40 other tropical shore species have transgressed the Barrier at least as far as the offshore islands of the Eastern Pacific. This gives a total of 53 shore fishes which can be called "trans-Pacific species." A list has been compiled (Appendix) which gives the name and the general distribution of each.

THE TRANS-PACIFIC SHORE FISHES

Although there is still a great deal to be learned about the life histories of the trans-Pacific shore fishes, it seems quite apparent that the majority must have crossed the East Pacific Barrier during the early stages of development. The seven species of eels presumably all have leptocephalid larvae, and both *Albula vulpes* and *Chanos chanos* are known to have larvae of a similar kind. The spiny larvae of some holocentrids are known so it is plausible that *Holotrachys lima* and *Myripristis murdjan* may have this type. *Zanclus cornutus* and *Forcipiger longirostris* probably have tholichthys larvae and the four surgeonfishes (of the genera *Acanthurus* and *Ctenochaetus*) undoubtedly have acronurus larvae.

It is likely that a few species made the journey across the Pacific as adults, particularly the six elasmobranchs on the list, for these forms no not have pelagic larvae and the adults attain an exceptionally large size. Also, a few of the plectognaths have been observed to venture away from the shore habitat. Some of these may obtain protection by accompanying floating debris.

THE DIRECTION OF MIGRATION

The probable direction of migration can be determined for those species where there is reasonable evidence to decide for or against an Eastern Pacific origin. It seems clear that those originating in one of the other tropical areas would have had to migrate across the East Pacific Barrier since the continental mass of the New World is probably a complete block to an approach from the east. This state of affairs has existed long enough (since the latest Pliocene or earliest Pleistocene when, according to Simpson 1950: 381, the Isthmus of Panama made its latest emergence) so that almost any of the Eastern Pacific shore fishes which did originate elsewhere, and are still identical with those of other areas, must

[1] Present address: Institute of Marine Science, Port Aransas, Texas.

have received relatively recent genetic reinforcement via migration of individuals from the west.

Aside from the 13 species of worldwide distribution, which possess an unusual ability to compete under a variety of environmental conditions, the great majority of the remaining 40 trans-Pacific forms have limited distributional patterns which indicate that they are of Indo-West Pacific origin. Current information available about the Eastern Pacific ranges of these species shows that 22 of them are confined to the offshore islands and that seven more are probably more typical of such areas than of the mainland, for they have been reported but once from the latter. Thus we have a total of 29 species, or about three-fourths of all the trans-Pacific forms with a non-worldwide distribution, either confined to, or probably most typical of, the offshore islands.

A strikingly similar distributional pattern for the trans-Pacific species of mollusks has been described by Hertlein (1937). Of the total of 18 in this category, the Eastern Pacific ranges of 13 are restricted to the offshore islands.

There is no reason to believe that oceanic islands do not have the same effect upon shallow water marine organisms that they do upon the terrestrial biota. That is, they function as evolutionary traps which weaken resident species rather than fitting them for the more rigorous competition of less isolated areas. As Darlington (1959) has pointed out, dominant groups evolve under the stress of a great variety of competition, not where it is reduced. Mayr (1942: 224) and Simpson (1953: 306) have spoken of the diminished variability developed in isolated, pioneer populations and Mayr (1954: 173) says that the evolutionist takes a "dim view" of the future prospects of such populations. In regard to the above mentioned 29 island inhabitants of the Eastern Pacific, one is tempted to assume that they came across from the Indo-West Pacific since the limited area and reduced competition of the island environment is hardly likely to produce a species that could migrate and successfully establish itself elsewhere.

Further indirect evidence for a western origin of the 29 species that are essentially confined, in the Eastern Pacific, to the offshore islands, may be found when the distributions of the genera represented by these species are examined. With the exception of the monotypic *Zanclus*, the genera concerned are all represented by a greater number of species in the Indo-West Pacific, indicating that this has probably been the primary region of generic development.

Evidence of this same kind is of some help in deciding whether or not any of the 13 worldwide species may have originated in the Eastern Pacific. Although four of the genera are monotypic (*Galeocerdo, Aetobatus, Albula*, and *Rachycentron*), the rest, judging from the species known at present, demonstrate that the greater part of the generic evolution has taken place in the Indo-West Pacific.

There remain but 11 trans-Pacific species that have not been discussed under one of the two foregoing categories. One, *Chanos chanos*, represents a monotypic genus, but the rest all belong to genera that are, again, better developed to the west. It is also interesting that three of them (*Echinorhinus cookei, Iniistius pavoninus*, and *Lactoria diaphana*) have been reported from single mainland localities only and must be regarded as rare rather than well-established in the Eastern Pacific.

Finally, of the total number of 53 tropical shore fishes which traverse the East Pacific Barrier, 40 demonstrate an extremely broad distribution to the west extending from Polynesia through the Indian Ocean and reaching the East African coast or its vicinity. In most cases, as can be seen by the list, this broad range to the west may be contrasted to what seems to be a limited and precarious foothold in the east.

In view of the foregoing information, plus the fact that there is not a single example of a species belonging to a typical New World genus gaining a foothold in Western Pacific,

even at its outermost fringes, it may be concluded that there is good circumstantial evidence for a western origin for the great majority of the trans-Pacific species. If this be accepted it can be said that there is, therefore, a recent (or current) invasion of the Eastern Pacific tropics from the west. This may be called "a phenomenon of eastward colonization."

Reasons for an Eastward Colonization

It is most intriguing that all available information should indicate an invasion of species across the East Pacific Barrier from the west when the predominant movement of surface water is in the opposite direction. The two great equatorial currents certainly carry many times the volume of the relatively narrow Equatorial Countercurrent. Yet, as Herre (1941) has pointed out, the latter stream must be the migratory pathway for the trans-Pacific forms. It should, of course, be assumed that fish larvae and occasional adults are carried in both directions, so that the important problem for the zoogeographer lies not in the details of the migratory movement itself but in the question of why it should result in colonization on one side and not the other.

Hubbs (1952) has stated that the paucity of the Panamanian fish fauna, particularly among the reef fishes, suggests widespread extermination by a marked cooling of the tropical Eastern Pacific. He also says that a decimated reef fauna would nicely explain the obviously very recent establishment on the tropical American Pacific Coast, particularly on the outlying islands, of Indo-Pacific fishes. Hubbs goes on to state that a decrease of about 8° C in the winter would account for the presumed annihilation, during the late Pleistocene, of much of the fish life of the Panamanian region.

More recently, Hubbs (1957) has assumed that the Pleistocene cooling of the Eastern Pacific was only about 3°C in the winter and his latest publication on this subject (1960) states that this assumption is in essential agreement with O^{18} temperature estimates from organisms in sea-bottom cores. In these works, Hubbs has also emphasized that the latest (Wisconsin) ice stage was outstandingly cold, very likely colder than the previous periods of Pleistocene glaciation.

If the eastward colonizations of Indo-West Pacific fishes have been made possible by the destructive effects of a late Pleistocene cooling, why have most of them taken place on the offshore islands rather than on the mainland coast? One would expect both areas to be affected by such cooling since it was apparently a worldwide phenomenon. The offshore islands of the Eastern Pacific are very small and, collectively, would stand far less chance of intercepting organisms borne by the Equatorial Countercurrent than would the mainland shore line. Even granted that most of the fish larvae so transported would need to settle down over a rocky substrate, there is still a vastly greater area of this habitat available along the mainland and about the onshore islands. In fact, if Hubbs' theory were correct, a reversal of the present distributional pattern in the Eastern Pacific might well be expected. That is, the colonizers would tend to be concentrated along the mainland coast and, once having attained it, would become widespread in the suitable warm water areas.

It is not surprising that a number of species from the west would be able to establish themselves in the New World, for this is a demonstration of a basic zoogeographic concept that received its modern emphasis from Matthew (1915)—that dominant species arise in certain important centers and gradually become dispersed into the peripheral areas. As Darlington (1959: 503–504) has shown so well, a very large, warm, stable region (such as the Indo-West Pacific) may be expected to promote the evolution of dominant groups which will then spread outward. Since many of the trans-Pacific shore fishes probably represent recently evolved, dominant species, they can invade other areas even in the face of the competition provided by an undisturbed ecosystem.

FIG. 1. Present extent of the Eastern Pacific Tropical (Panamanian) Faunal Region.

It follows that it would be even easier for migrants from the Indo-West Pacific to settle around the offshore islands, because these represent areas of less competition than the Eastern Pacific mainland. First, the total number of species is markedly lower about the islands, and, second, a large percentage of those shore species that are present demonstrate, morphologically, the effects of isolation. Presumably, because of genetic loss, those in the latter category would not only have reduced variation but would have a lowered resistance to invasion, since such forms are known to be exceedingly vulnerable to extermination (Mayr, 1942: 224, 1954: 173).

There is considerable doubt that a drop of 3° C in the sea-surface temperature in the winter, such as Hubbs (1960: 106) calculated for the Wisconsin glaciation, would be sufficient to produce a widespread extermination of the Panamanian fish fauna. The latitudinal boundaries of the Eastern Pacific Tropical (Panamanian) Faunal Region are located roughly in the vicinity of the winter 20° C surface isotherm (calculated from the February mean in the northern hemisphere and the August mean in the southern hemisphere). Utilizing the data provided by Sverdrup, Johnson, and Fleming (1946: charts II and III), it can be seen that the tropical shelf fauna presently extends from the lower, outer coast of Baja California and the Gulf of California to about Cape Aguja, Peru (fig. 1). If the winter mean surface temperature of the entire area were lowered 3° C, it would certainly cause some latitudinal constriction of the tropical region (fig. 2) but would scarcely threaten its existence. This would, in fact, bring the winter mean of the Costa Rican-Panamanian area to only about 25° C, a temperature still warm enough to support a rich, characteristic tropical fauna.

The distributional map recently published by Wells (1957: pl. 9) reveals very little coral reef development in the whole of the tropical Eastern Pacific. The scarcity

FIG. 2. Approximate extent of the Tropical Faunal Region with the mean winter temperature lowered 3° C.

may be due to upwelling water or lack of bedrock as was suggested by Ekman (1953: 4) but, whatever the cause, it seems logical to suppose that a poor representation of reef coral should also mean an impoverished associated fauna, including fishes.[2]

In summary, it can be stated that Hubbs' theory of a late Pleistocene catastrophe in order to explain the paucity of the Panamanian fish fauna and the eastward colonization of certain Indo-West Pacific species seems to be unnecessary. The distributional pattern of the species from the west is better explained on the basis of the dispersal of dominant forms into peripheral areas which have relatively undisturbed ecosystems, and there is a poor representation of reef fishes because very little of the proper habitat is available. In addition, there is doubt that the late Pleistocene (or at least the Wisconsin glaciation) temperatures were cold enough to bring about a widespread extermination in the tropical Eastern Pacific.

[2] The intimate nature of the association of many of the Western Pacific fishes with the coral reef habitat, is beautifully shown by Hiatt and Strasburg (1960).

EFFECTIVENESS OF THE BARRIER

Ekman (1953: 73) has said "This oceanic region, the *East Pacific Barrier*, is responsible for the most pronounced break in the circumtropical warm-water fauna of the shelf. This is particularly true of the distribution of species and genera and to a certain extent also for that of families." This statement has been referred to by almost every marine zoologist who has recently taken occasion to write about the broad aspects of distribution in the Pacific. However, there needs to be a fundamental qualification made as far as the shore fishes are concerned.

As more systematic work is done on the families and genera of amphi-American distribution, the species which can be rec-

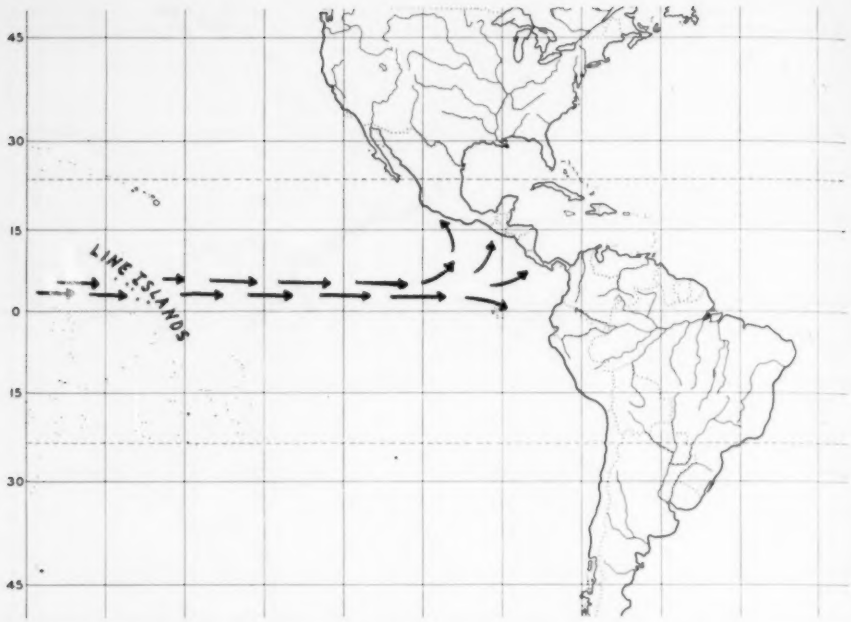


FIG. 3. The position of the Line Islands and the course of the Equatorial Countercurrent (after Sverdrup, Johnson, and Fleming, 1946—modified).

ognized as common to both the Eastern Pacific and Western Atlantic become fewer. In fact, the most recent studies along this line such as the revisions of the Scaridae (Schultz, 1958), the Antennariidae (Schultz, 1957), the clinid genera *Labrisomus* and *Malacoctenus* (Springer, 1958), and the Gobiesocidae (Briggs, 1955), show no amphi-American species whatsoever!

As far as the species level is concerned, it should now be recognized that the New World land mass constitutes a barrier that is virtually complete. Aside from the 13 species of circumtropical shore fishes and a few euryhaline forms which could negotiate the Panama Canal or migrate by means of fresh-water streams, there are probably less than a dozen shore fishes common to the tropical waters on both sides of the Isthmus of Panama. These can best be considered as examples of forms in which evolutionary change has taken place so slowly that divergence to the species level has not been attained.

It is necessary, then, to qualify Ekman's observation by the following statement: The distribution of tropical shore fishes demonstrates that it is the *Land Barrier* comprising the continental mass of the Western Hemisphere that is responsible for the most pronounced break in the circumtropical distribution of *species*, not the East Pacific Barrier. The amphi-American occurrence of so many genera (and families) is unquestioned and is, of course, attributable to the extended submergence of the Panamanian isthmus from the Paleocene through at least most of the Pliocene. The East Pacific Barrier is, without doubt, the most important of all for these higher (and older) categories.

A rough estimate of the degree of effectiveness of the East Pacific Barrier can be made by taking into consideration the num-

ber of shore fish species that are theoretically available for transport across the Pacific and comparing it to the number that apparently have successfully made the journey. This assumes that effective migration takes place only from west to east, and that the means of transport is the Equatorial Countercurrent.

It can be observed (fig. 3) that the Line Islands are the easternmost Polynesian group that lie along the course of the Equatorial Countercurrent. Unfortunately, the fish fauna of this archipelago is very poorly known. However, one might expect to find at least as many shore fishes as have been recently reported from Hawaii by Gosline and Brock (1960) — 387. Since 53 (the shore fishes that are apparently trans-Pacific in range) is 13.7% of 387, and 100%–13.7% is 86.3%, this latter figure gives, at least, an approximation of the efficiency of the East Pacific Barrier in regard to the distribution of shore species. In comparison, the efficiency of the New World Land Barrier may be similarly reckoned at roughly 98.0%.

Literature Cited

de Beaufort, L. F. 1940. The fishes of the Indo-Australian Archipelago. Vol. 8. E. J. Brill, Leiden. 508 p.

Briggs, J. C. 1955. A monograph of the clingfishes (Order Xenopterygii). Stanford Ichthyol. Bull., **6**, 224 p.

——. 1960. Fishes of worldwide (circumtropical) distribution. Copeia, **1960**: 171–180.

Darlington, P. J. 1959. Area, climate, and evolution. Evolution, **13**: 488–510.

Darwin, Charles. 1872. The origin of species. 6th Ed. Appleton, New York. Vol. 2, 338 p.

Ekman, Sven. 1953. Zoogeography of the sea. Sidgwick and Jackson, London. 417 p.

Garrick, J. A. F. 1960. Studies on New Zealand Elasmobranchii. Part X—the genus *Echinorhinus*, with an account of a second species, *E. cookei* Pietschmann, from New Zealand waters. Trans. Roy. Soc. New Zealand, **88**: 105–117.

Gosline, W. A., and V. E. Brock. 1960. Handbook of Hawaiian fishes. Univ. Hawaii Press, Honolulu. 372 p.

Herre, A. W. 1941. Distribution of fish in the tropical Pacific. Proc. 6th Pacific Sci. Congress, **3**: 587–592.

Hertlein, L. G. 1937. A note on some species of marine mollusks occurring in both Polynesia and the western Americas. Proc. Am. Philos. Soc., **78**: 303–312.

Hiatt, R. W., and D. W. Strasburg. 1960. Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol. Monogr., **30**: 65–127.

Hubbs, C. L. 1952. Antitropical distribution of fishes and other organisms. Proc. Seventh Pacific Sci. Congr., **3**: 324–329.

——. 1957. Recent climatic history in California and adjacent areas. Proc. Conf. Recent Research in Climatology, La Jolla, Calif., pp. 10–22.

——. 1960. Quaternary paleoclimatology of the Pacific coast of North America. Rept. Calif. Coop. Ocean. Fisheries Invest., **7**: 105–112.

Lacépède, B. G. E. 1803. Histoire naturelle des poissons. Vol. 5. Chez Plasson, Paris. 803 p.

Matthew, W. D. 1915. Climate and evolution. Ann. New York Acad. Sci., **24**: 171–318.

Mayr, Ernst. 1942. Systematics and the origin of species. Columbia Univ. Press, New York. 334 p.

——. 1954. Change of genetic environment and evolution. *In* Evolution as a process. (Edited by J. Huxley, A. C. Hardy, E. B. Ford). George Allen and Unwin, London. 367 p.

Randall, J. E. 1955. Fishes of the Gilbert Islands. Atoll Research Bull. No. 47. Pacific Science Board, Washington, D. C., 243 p.

Schultz, L. P. 1957. The frogfishes of the family Antennariidae. Proc. U. S. Nat. Mus., **107**: 47–105.

——. 1958. Review of the parrotfishes, family Scaridae. U. S. Nat. Mus. Bull., **214**: 143 p.

Simpson, G. G. 1950. History of the fauna of Latin America. Am. Sci., **38**: 361–389.

——. 1953. The major features of evolution. Columbia Univ. Press, New York. 434 p.

Springer, V. G. 1958. Systematics and zoogeography of the clinid fishes of the subtribe Labrisomini Hubbs. Inst. Mar. Sci., Texas, **5**: 417–492.

Storey, M. H. 1939. Contribution toward a revision of the ophichthyid eels. I. The genera *Callechelys* and *Bascanichthys*, with descriptions of new species and notes on *Myrichthys*. Stanford Ichthyol. Bull., **1**:61–84.

Sverdrup. H. U., M. W. Johnson, and R. H. Fleming. 1946. The oceans. Prentice-Hall, New York. 1087 p.

Wells, J. W. 1957. Coral reefs. *In:* Treatise on marine ecology and paleoecology, vol. 1, ecology (Edited by J. W. Hedgpeth). Geol. Soc. Am. Mem., **67**, 1296 p.

Woods, L. P., and L. P. Schultz. 1953. Family Hemiramphidae. *In:* Schultz and collaborators, Fishes of the Marshall and Marianas Islands. U. S. Nat. Mus. Bull., **202**, 685 p.

Appendix

List of Trans-Pacific Shore Fishes

1. *Galeocerdo cuvieri* (Lesueur). Tiger shark

Southern California to Ecuador and Cocos, Clipperton and the Galapagos Islands. Hawaii and Japan south to New Zealand, westward to Africa, and both sides of the Atlantic.

2. *Echinorhinus cookei* Pietschmann. Spiny shark

One Eastern Pacific record from Southern California. Hawaii south to New Zealand.

3. *Triaenodon obesus* (Rüppell)

Panama and Cocos Island. Hawaii and the Philippines south to the New Hebrides and west to Africa.

4. *Sphyrna tiburo* (Linnaeus). Bonnethead

Southern California to Ecuador. Reported from the Philippines and China and from both sides of the Atlantic.

5. *Sphyrna zygaena* (Linnaeus). Smooth hammerhead

Southern California to Chile and the Galapagos Islands. Japan south to New Zealand and westward to Africa. Also on both sides of the Atlantic.

6. *Aetobatus narinari* (Euphrasen). Spotted eagle ray

Northern California to Panama and the Galapagos Islands. Hawaii and Japan, south to Australia, west to Africa, and on both sides of the Atlantic.

7. *Albula vulpes* (Linnaeus). Bonefish

Northern California to Panama. Hawaii and Japan, south to the Tuamotus and Australia, westward to Africa, and both sides of the Atlantic.

8. *Chanos chanos* (Forskål). Milkfish

Baja California to Panama and the Galapagos Islands. Hawaii and Japan, south to the Tuamotus and New Zealand, and west to Africa.

9. *Echidna zebra* (Shaw)

A specimen from the Tres Marias Islands is in the U.B.C. collection (BC60-507); taken by Richard Krejsa on March 19, 1960. Hawaii and the Philippines, south to Australia, and west to Africa.

10. *Gymnothorax pictus* (Ahl)

Revillagigedo Archipelago. Hawaii and the Riu Kiu Islands, south to the Tuamotus and Australia, and west to Africa.

11. *Gymnothorax chilospilus* Bleeker

Galapagos Islands. Philippines to Sumatra and out to Samoa and Tahiti.

12. *Gymnothorax undulatus* (Lacépède)

Galapagos Islands. Hawaii and the Philippines, south to the Tuamotus and Australia, and west to Africa.

13. *Callechelys marmoratus* (Bleeker)

Galapagos Islands. Marshall Islands to Sumatra and west to Africa. Storey (1939) indicates that the Galapagos specimens may be referable to *C. luteus* Snyder, a Hawaiian endemic. From a distributional standpoint, this seems unlikely unless the latter actually has a wider range in the Western Pacific.

14. *Brachysomophis crocodilinus* (Bennett)

Galapagos Islands. Japan to Tahiti and west to Mauritius and Aldabra.

15. *Myrichthys maculosus* (Cuvier)

Galapagos Islands. Hawaii and Guam, south to New Guinea and west to Africa.

16. *Belone platyura* Bennett

Galapagos, Revillagigedos, and Cape San Lucas, Baja California to Panama. Hawaii and Guam, south to Easter Island and Australia, and west to Africa. *B. persimilis* Günther is considered to be a synonym.

17. *Euleptorhamphus viridis* (van Hasselt). Ribbon halfbeak

Galapagos, Revillagigedos, and a record from California. Hawaii and Marshall Islands, south to Australia, and west to Africa. This species has usually been called *E. longirostris* (Cuvier) but Woods and Schultz (1953) have shown that it is a synonym of the above.

18. *Holotrachys lima* (Valenciennes)

Cocos Island. Hawaii, Marshall, and Gilbert Islands to Samoa and westward to Africa.

19. *Myripristis murdjan* (Forskål)

Galapagos and Cocos Islands. Marshall and Gilbert Islands to Japan, south to the Tuamotus and New Guinea, and west to Africa.

20. *Priacanthus cruentatus* (Lacépède). Glasseye snapper

Cape San Lucas, Baja California, to Panama Bay and the Galapagos, Cocos, and Revillagigedo Islands. Hawaii and Japan to Tahiti, west to Africa, and both sides of the Atlantic.

21. *Aulostomus chinensis* (Linnaeus). Trumpetfish

Cocos and Revillagigedo Islands. Hawaii and Japan, south to the Tuamotus and Australia, and westward to Madagascar. Specimens from both Eastern Pacific localities are in the U.B.C. collection.

22. *Fistularia petimba* Lacépède

Baja California to Panama, the Galapagos, and Revillagigedos Islands. Hawaii and Japan, south to the Tuamotus and Australia, and west to Africa.

23. *Doryrhamphus melanopleura melanopleura* (Bleeker)

Baja California to Colombia and the Galapagos Islands. Marshall Islands to Cocos Island (in the Eastern Indian Ocean).

24. *Doryrhamphus melanopleura pleurotaenia* (Günther)

Revillagigedo Archipelago. Hawaii and Okinawa to the Solomon Islands.

25. *Kuhlia taeniura* (Cuvier)

Baja California to Colombia and the Galapagos, Cocos, and Revillagigedos Islands. Gilbert and Marshall Islands to Japan, south to Lord Howe Island, and westward to Africa.

26. *Lutjanus kasmira* (Forskål)

Galapagos Islands. Gilbert and Marshall Islands to Japan, south to the Tuamotus and Australia, and west to Africa.

27. *Rachycentron canadus* (Linnaeus). Cobia

Northern coast of Chile. Japan south to Australia, west to Africa, and both sides of the Atlantic.

28. *Runula tapeinosoma* (Bleeker)

Galapagos Islands. Gilbert Islands to the New Hebrides and west to Africa. This species has most commonly been referred to the genus *Petroscirtes*.

29. *Zanclus cornutus* (Linnaeus). Moorish Idol

Cape San Lucas, Baja California, and the Galapagos, Cocos, and Revillagigedo Islands. Hawaii and Japan, south to the Tuamotus and Australia, and west to Africa. I include the nominal species *Z. canescens* (Linnaeus) which is apparently based on immature specimens.

30. *Forcipiger longirostris* (Broussonet). Longbeak butterflyfish

Revillagigedo Archipelago. Hawaii and Japan, south to the Tuamotus and Australia, and west to Africa.

31. *Abudefduf saxatilis vaigiensis* (Quoy and Gaimard). Sergeant major

Galapagos Islands. Gilbert Islands and Japan, south to the Tuamotus and Australia, and west to Africa. de Beaufort (1940) considers this subspecies distinct from that (*A. s. saxatilis*) found along the mainland coast of the Eastern Pacific and in the Atlantic.

32. *Iniistius pavoninus* (Valenciennes)

Cape San Lucas, Baja California. Hawaii south to Lord Howe Island and west to Bali.

33. *Thallasoma lutescens* (Lay and Bennett)

Galapagos, Tres Marias, and Revillagigedos Islands. Hawaii and Japan south to Australia.

34. *Scarops jordani* (Jenkins)

Acapulco, Mexico, and Cocos and the Revillagigedos Islands. Hawaiian Islands.

35. *Mugil cephalus* Linnaeus. Striped mullet

Northern California to Chile and the Galapagos Islands. Hawaii and Japan, south to New Zealand, west to Africa, and both sides of the Atlantic.

36. *Acanthurus triostegus triostegus* (Linnaeus)

Baja California to the Tres Marias Islands and the Galapagos, Cocos, Clipperton, and Revillagigedos Islands. Line Islands and Japan, south to the Tuamotus and Australia, and west to Africa.

37. *Acanthurus glaucopareius* Cuvier

Galapagos, Cocos, Clipperton, and Revillagigedos Islands. Hawaii and Japan, south to New Guinea, and west to the Eastern Indian Ocean.

38. *Acanthurus xanthopterus* (Valenciennes)

Northern Mexico to Panama and the Galapagos Islands. Hawaii and the Philippines, south to Samoa, and west to Africa.

39. *Ctenochaetus cyanoguttatus* Randall

Cocos Island. Line Islands to the Marquesas and west to Aldabra in the Western Indian Ocean.

40. *Canthidermis maculatus* (Bloch). Smooth triggerfish

Galapagos and Cocos Islands. Hawaii and Japan, south to New Guinea and possibly Australia, west to Africa, and both sides of the Atlantic. Includes *C. rotundatus* (Proce) and *C. angulosus* (Quoy and Gaimard).

41. *Xanthichthys ringens* (Linnaeus). Redtail triggerfish

Southern California to Panama and the Revillagigedo Islands. Hawaii and Japan, south to Easter Island and New Guinea, west to Africa, and both sides of the Atlantic. *X. lineopunctatus* (Hollard) is considered a synonym.

42. *Melichthys radula* (Solander). Black durgon

Cocos, Malpelo, and Revillagigedo Islands. Hawaii and Japan, south to Tuamotu Islands, west to Africa, and both sides of the Atlantic. This species has recently been called *M. buniva* (Lacépède) by Randall (1955) and Gosline and Brock (1960). However, the latter name was applied by Lacépède (1803: 669, pl. 21) to a Mediterranean specimen (Nice), and has for many years been considered a synonym of the common Atlantic species *Balistes capriscus* Gmelin. There is no reason to suspect that this action was incorrect.

43. *Alutera monoceros* (Osbeck). Unicorn filefish

Mazatlán, Mexico, and the Revillagigedos Islands. Hawaii and Japan, south to New Zealand, west to Africa, and both sides of the Atlantic.

44. *Alutera scripta* (Osbeck). Scrawled filefish

West coast of Mexico to Panama and the Revillagigedos Islands. Hawaii and Japan, south to Australia, west to Africa, and both sides of the Atlantic.

45. *Amanses carolae* (Jordan and McGregor)

Revillagigedos Islands. Hawaii and Gilbert Islands.

46. *Arothon hispidus* (Linnaeus)

Costa Rica to Panama and Clipperton Island. Hawaii and Japan, south to the Tuamotus and Australia, and west to Africa.

47. *Diodon holacanthus* Linnaeus. Balloonfish

Baja California to Colombia and the Galapagos Islands. Hawaii to Japan, south to Easter Island and Australia, west to Africa, and both sides of the Atlantic.

48. *Diodon hystrix* Linnaeus. Porcupinefish

Southern California to Chile and the Galapagos and Revillagigedos Islands. Hawaii and Japan, south to the Tuamotus and New Zealand, west to Africa, and both sides of the Atlantic.

49. *Chilomycterus affinis* Günther. Pacific burrfish

Southern and Baja California and the Galapagos Islands. Across the northern tropical Pacific from Hawaii to Japan.

50. *Ostracion meleagris* Shaw

Santiago Bay, Mexico, and the Galapagos, Clipperton, and Revillagigedos Islands. Hawaii and Japan, south to the Tuamotus and Australia, and west to Africa. *O. lentiginosus* Bloch and Schneider and *O. sebae* Bleeker are synonyms.

51. *Lactoria diaphana* (Bloch and Schneider). Spiny trunkfish

Southern California. Hawaii and Japan, south to Tasmania, and west to Africa.

52. *Antennatus bigibbus* (Lacépède)

Revillagigedo Islands. Hawaii to the Tuamotu Islands, to Australia, and west to Africa.

53. *Antennarius drombus* Jordan and Evermann

Cocos Island. Hawaiian Islands.

TESTS FOR SOCIAL DISCRIMINATION BETWEEN THREE SPECIES OF THE *PEROMYSCUS TRUEI* SPECIES GROUP OF WHITE-FOOTED MICE[1]

J. R. Tamsitt[2]
Department of Zoology, University of Texas, Austin, Texas

Received March 27, 1961

Perhaps because of the cryptic nature of ethological isolating mechanisms in small mammals, behavioral and social differences between individuals and groups of species of small rodents have received little attention. Ethological factors which restrict random mating between closely related forms are well known to ornithologists (see Mayr, 1949), and Dice (1940) has emphasized that sexual isolation is one of the most important factors in the speciation of mammals. This stage of speciation is correlated with data indicating that, in general, reproductive isolation through ethological or social factors precedes the development of intersterility in mammals.

To determine the existence of social isolation in artificial societies of *Peromyscus*, Blair and Howard (1944) devised an experimental method which was later applied by several workers (Blair, 1953, 1954; Clark, 1952; McCarley, 1953; Bradshaw, 1956) to various species of this genus. As data given by these authors indicate the existence of social isolation between *Peromyscus* species, this experimental method was applied to the sibling species *Peromyscus comanche*, *P. nasutus*, and *P. truei* of the *Peromyscus truei* species group.

Methods

Experimental Conditions.—To create a simple artificial society of two pairs of mice, a method was utilized in which a choice of mating partners was offered. A cage constructed of galvanized sheet metal and ¼″ mesh hardware cloth measuring 5½″ high with floor dimensions of 30 × 15″ was divided linearly into four compartments by wire mesh partitions. Inserted in the partitions were short wooden tunnels which allowed the mice access to any compartment. Cotton nesting material and a water bottle were made available in each compartment, and a balanced ration of standard commercial laboratory pellets was constantly supplied to the mice.

It was possible to regulate the environment of the laboratory to a limited extent. Experiments were conducted in a room in which the temperature varied little from a mean of 70° F throughout the year, and the room was darkened at least eight hours during the night.

Source of Stocks.—The mice tested represented two wild populations of *Peromyscus comanche*, five of *P. nasutus*, and three of *P. truei*. The *comanche* were obtained from Randall County, Texas, and the stocks of *nasutus* and *truei* from Lincoln County, New Mexico.

Laboratory Routine.—The wild-trapped stocks of *Peromyscus* were returned from the trapping sites to a vertebrate animal room at The University of Texas. Females were caged alone and held in cages for at least a month prior to their use in the tests. The isolation and retention of the females for this period prevented the use of pregnant mice in a new experiment. Males were caged alone or temporarily with another male. All mice were housed in ventilated cages similar to those recommended by Dice (1929).

Each experiment was initiated by placing the two pairs of mice to be tested in one of the end compartments of a clean cage. Only mice of breeding age were used. To prevent bias resulting from familiarity between individuals, the same combination of four mice was never repeated in later experiments. Notches in various combinations

[1] Based on a dissertation submitted in partial fulfillment for the degree of Doctor of Philosophy, Department of Zoology, University of Texas, January 1958.

[2] Present address: Department of Biology, University of the Andes, Bogotá, Colombia.

were punched in one or both ears to identify individual mice in any single experiment.

Unless interrupted by the death of an individual or the birth of a litter, each experiment was continued 30 days. Exceptions were experiments terminated due to the severe wounds of an individual and indication of his impending death. Thirty days was the testing period chosen by Clark (1952), for this duration exceeded by approximately one-third the gestation period of the species involved. Svihla (1932) indicated the gestation period of *truei* to vary from 25 to 40 days, but the latter period was considered exceptional. After an experiment was terminated, the females were isolated and little disturbed for 30 additional days to insure survival of any young from experimental matings. A duration of 30 days sufficed for the establishment of a social pattern, and a lapse of 60 days, from inception of an experiment to removal from isolation, was sufficient for parturition.

In nature *Peromyscus* is primarily nocturnal. In captivity, locomotor activity began shortly after the room was darkened at night and continued with decreasing intensity until daybreak. At this time they became temporarily or permanently arranged in one of 15 possible social combinations. Each experimental cage was inspected between 8 and 10 AM, and the daily distribution of the mice in the compartments of each cage was recorded. These records comprise the data recorded in table 1. Other evidence of social behavior, such as wounds from sporadic fighting between mice, were likewise recorded at the time the spatial positions were noted.

Treatment of Data.—For each quartet of mice associated in an experiment, 15 different spatial combinations are possible. These combinations express in general four types of behavior: 1) species discrimination, 2) absence of species discrimination, 3) dominance, and 4) gregariousness.

The most critical of these are three categories indicative of species discrimination. These include pairings of one male and one female of the same species and simultaneous pairings of the four mice by species. If equal probabilities for occupation of the four compartments are assumed, these combinations could occur by chance alone 23% of the time (see Blair and Howard, 1944, for derivation of probabilities). Opposed to these pairing scores is the second group of combinations involving cross-pairs. These combinations include pairings of a male of one species with females of the opposite species. These pairings are regarded as evidence against species discrimination, and would likewise occur by chance in 23% of the observations.

The third general type are classes in which three mice nest together to the exclusion of the fourth. This spatial combination occurs most frequently when one male is excluded, and is interpreted as dominance of one male over the other. Groups of three could occur on 19% of the days as a result of random association. The fourth category involves the aggregation of all four individuals in a single compartment. This recorded behavior is evidence of gregariousness, and would occur by chance in 2% of the observations. Four other spatial combinations are possible (see table 1), but none of these is critical for interpreting social discrimination.

A chi-square test to determine the goodness of fit of the actual data to the theoretical distribution has been applied to compare combinations of equal probability. Likewise a formula premitting one degree of freedom was employed in testing a 1:1 ratio for all occurrences that have the same expected frequencies. In the chi-square formula, the deviation from the expected may be tested to determine the significance. This method is identical with that of Blair and Howard (1944), and a detailed discussion of the formulae and their use is given by Bradshaw (1956).

The Social Tests

comanche with *comanche*

Four hundred and twenty-two observations were obtained from tests composed of pure populations of *comanche* (table 1). Twenty tests were conducted, and 10 of

TABLE 1. *Social preference in intraspecific and interspecific tests with the species* Peromyscus comanche, P. nasutus, *and* P. truei

		Number observed	Double pairs	Male *a* with female *a*	Male *b* with female *b*	Female *a* out	Female *b* out	Male *a* out	Male *b* out	None together	Males together	Females together	All together	Males together, females together	Male *a* with Female *b*	Male *b* with female *a*	Double cross-pairs
comanche with *comanche*	Totals	422	22	14	9	8	18	49	87	6	12	41	65	42	20	6	23
	Expected	423.3	19.8	39.7	39.7	19.8	19.8	19.8	19.8	39.7	39.7	39.7	6.8	19.8	39.7	39.7	19.8
	Chi-square	—	0.3	16.6	23.7	7.0	0.2	43.1	228.1	28.6	19.3	0.04	494.7	24.8	9.7	28.6	0.5
nasutus with *nasutus*	Totals	525	12	4	4	39	40	39	59	3	10	15	242	27	10	6	15
	Expected	527.1	24.7	49.4	49.4	24.7	24.7	24.7	24.7	49.4	49.4	49.4	8.4	24.7	49.4	49.4	24.7
	Chi-square	—	6.5	41.7	41.7	8.2	9.4	8.2	47.6	43.5	31.4	23.9	6496.3	0.2	31.4	38.1	3.8
truei with *truei*	Totals	488	8	2	4	14	9	60	60	4	8	18	203	69	9	9	11
	Expected	489.4	22.9	45.9	45.9	22.9	22.9	22.9	22.9	45.9	45.9	45.9	7.8	22.9	45.9	45.9	22.9
	Chi-square	—	9.7	84.1	76.6	3.4	8.4	60.1	60.1	38.2	31.2	16.9	4885.0	92.8	29.6	29.6	6.1
comanche (*a*) with *nasutus* (*b*)	Totals	567	17	10	5	47	33	78	73	1	5	7	250	10	11	10	10
	Expected	569.1	26.7	53.3	53.3	26.7	26.7	26.7	26.7	53.3	53.3	53.3	9.1	26.7	53.3	53.3	26.7
	Chi-square	—	3.5	35.1	43.6	15.4	1.4	98.5	82.1	51.3	43.6	40.2	6377.2	10.4	33.5	35.1	10.4
comanche (*a*) with *truei* (*b*)	Totals	435	13	3	6	15	24	56	95	0	13	15	143	17	7	8	20
	Expected	436.8	20.5	40.9	40.9	20.5	20.5	20.5	20.5	40.9	40.9	40.9	7.0	20.5	40.9	40.9	20.5
	Chi-square	—	2.7	35.1	29.7	1.4	0.6	61.4	270.7	0.0	19.0	16.4	2642.2	0.6	28.1	26.4	0.01
nasutus (*a*) with *truei* (*b*)	Totals	550	19	14	8	35	43	70	67	4	7	11	231	12	10	8	11
	Expected	552.0	25.9	51.7	51.7	25.9	25.9	25.9	25.9	51.7	51.7	51.7	8.8	25.9	51.7	51.7	25.9
	Chi-square	—	1.9	27.4	36.9	3.1	11.2	75.1	65.2	44.0	38.6	32.0	5610.5	7.4	33.6	31.9	8.6

these were terminated before the standard 30 day test period. The birth of young to a female ended two tests, and six were concluded when a male was killed by a rival male. Two tests were ended when an individual died of apparently natural causes.

In seven of the 15 possible categories the number expected on the basis of chance distribution of the mice was exceeded by the observed totals, but when the chi-square test was applied to three of these seven categories, the observed totals did not significantly exceed the expected number. In four categories, however, the disparity between the actual and expected frequencies was too large to be attributed to chance. Whereas on the basis of chance a male was expected to be out of the aggregation 4.7% of the time, the *comanche* male *a* was out 11.5% of the time and the *comanche* male *b* 20.6%. Aggregations of all mice occurred 15.4% of the time, when only 1.6% were expected. Simultaneous unisexual pairings were observed 10.0% of the time, whereas only 4.7% were expected by chance.

The totals of all observed single pairs and cross-pairs were considerably less than the frequencies expected. When these two categories were compared with the 1:1 ratio, the chi-square value of their difference was 0.2, which is not significant. Similarly the difference between the number of double pairs and double cross-pairs was not significant (chi-square = 0.1). The lumped scores of all pairings and all cross-pairings differed only slightly. The total observed number of single and double pairings comprised 10.7% of the 422 observations, whereas 11.6% were composed of single and double cross-pairings. In general, pairings in a pure population of *comanche* did not occur more frequently than would be expected on the basis of chance distribution.

Examination of the frequencies of a male with one female or the other in all categories in which this arrangement could occur revealed to some extent the polygamous behavior of the males in the test situation. The *comanche* male *a* was in the same compartment with one female 48.8% of the time and with the other 48.1%. The *comanche* male *b*, on the other hand, was with one female 36.3% of the time and with the other 38.2%.

In 38.4% of the observations an individual was absent from the aggregation. Females, however, were more often members of the nesting trio than were males. A female was "out" 6.2% of the time, whereas one or the other males was excluded 32.2%.

Gregariousness, i.e., when the four members nested together in one compartment, was noticed in 15.4% of the observations but was conspicuous only in three experiments. Although the actual number of quartet aggregations was significantly greater than expected, this spatial arrangement was less common in this series than in the other five test series.

Domination of a male by the other accounted for the death of six individuals and the wounding of seven others. In the tests in which physical manifestations of dominance were noticed, the defeated male nested separate from the aggregation a significant number of times.

nasutus with *nasutus*

Fourteen of the 20 tests with this species were terminated at the end of the 30 day test period, whereas two were ended when a male was killed by another male, three when young were born to a female, and one when a female died of natural causes. Five hundred and twenty-five observations were accumulated. A summary of the results is given in table 1.

Only one of the 15 possible combinations did not differ significantly from the expected. This category was that in which the males nested together and the females nested together simultaneously. Several other categories differed considerably from the expected probability. These were single pairings, single cross-pairings, one male "out," no mice together, males together, and aggregations of all four mice.

The most commonly observed spatial arrangement was the quartet aggregation (46.1% of the observations), and the high frequency of this arrangement is indicative

of the gregariousness of individuals of this species in a pure society. Pairing and cross-pairing frequencies were low, and the observed totals of all categories of this kind were considerably less than expected. Likewise when all categories were considered, the frequency of the association of one male with one or the other female revealed no difference in preference. Trio aggregations, with the fourth member nesting separately, comprised 22.9% of the 525 observations. Although a female was out of the aggregation almost as often as a male, there was no significant difference between the number of times an individual of either sex nested separately. Other comparisons of categories with the 1:1 ratio gave no significant difference.

In these tests the number of deaths was low, and only two males died of fatal wounds. The few fatalities and the low incidence of wounds exhibited by individuals in these tests indicate that for the most part mice of this species live harmoniously with each other in the test situation.

truei with *truei*

A summary of the data from each of the 20 *truei* tests is given in table 1. Comparison of the totals reveals that the expectancies based on chance distribution were exceeded in four of the 15 possible categories. These occurred when one male was "out," aggregations of all four mice, and when the sexes were paired at the same time. One of the males was out of the aggregation 24.5% of the time, whereas a female was recorded as "out" only 4.7%. Aggregations of all four mice occurred 41.6% of the time when only 1.6% were expected. Although the occurrence of males together and females together at separate times was considerably lower than the expected, the percentage of simultaneous unisexual pairings (14.1%) considerably exceeded the expected (4.7%).

Double pairings were seen in 1.6% of the observations, with 4.6% expected. Double pair scores totaled eight, and double cross-pairs totaled 11. These were not significant deviations. The incidence of simple pairing and simple cross-pairing was considerably below that expected. Only 1.2% of the 488 daily records were those of simple pairing, while 3.7% were those of simple cross-pairing. The high incidence of three categories, those of a male "out," simultaneous unisexual pairings and quartet aggregations, and the low frequencies of pairing and cross-pairing, indicate that the daily distribution of the individuals was not based on chance. Supporting this premise is the lower total of all other observed categories when compared with the expected probability. An examination of categories in which a male was with one or both females revealed that a male was with one or the other female 60% of the time. These data, when considered with the low frequencies of pairing observed, indicate that pairing tendencies are lacking in a pure society of *truei*.

Fighting of some kind, resulting in minor or major wounds or death, was evident in half of the tests. Injuries inflicted were primarily to the rump, tail, eyes, head or ears. Complete male dominance was noticed in two experiments. In one a male was killed the first day, and in another the death of a male occurred on the second day. In two other experiments male dominance was not conspicuous in the observed totals, but a male was killed in each.

comanche with *nasutus*

The 20 tests reported here are summarized in table 1. The expectancies based on chance distribution were exceeded by the observed totals in three categories. One, in which the mice nested together, comprised 44.1% of the observations, and is indicative of the sociability of these two species in the artificial environment. In another category a male was out of the assemblage in 26.6% of the observations. The *comanche* male was "out" 13.7% of the time, a significant difference from the 4.8% expected. Similarly the *nasutus* male was out of the aggregation 12.9% of the time, whereas the expected percentage was 4.8. Although males of both species were out more often than can be ex-

plained by chance, each was out approximately the same amount of time. The third category in which the observations exceeded the expectations was that in which one female was "out." On the basis of chance one female would have been out in 9.3% of the 567 observations. The *nasutus* female was "out" 5.8% of the time, and the *comanche* female was "out" 8.3%. Although the difference between the times these two females were excluded is not significant, the number of times that a male was out differs significantly from the number of times that a female was out.

If all categories of cross-pairing are compared with those in which there was species pairing, it is found that these approximate each other. When the four mice separated from the aggregation in which they most frequently nested, they did not pair by species a significantly greater number of times than when they nested in cross-pair combinations. The number of species double pairings (3.0%) was greater than the number of double cross-pairings (1.8%), but the difference between the two categories is not significant. Likewise, the minor differences between the number of simple pairs and simple cross-pairs indicate there is little social isolation between these two species. Although species pairing did occur, gregariousness appeared to be the preference in the artificial environment.

Male dominance in these tests was not pronounced. A male was "out" 26.6% of the time, and although this occurred a significant number of times, there was no significant difference between the number of times that a male of one or the other species was out. When considered alone, the totals indicate an equally dominant role for males of both species. Likewise fighting was less evident with this species combination than with any other. No males were killed, and only in four experiments were males wounded.

comanche with *truei*

Several categories in tests with these species exceeded the expected probability (table 1). Aggregations of all four mice comprised 32.9% of the 435 daily observations. The number of times one male nested with the two females to the exclusion of the other male was outstanding. This occurred 34.7% of the time when only 9.4% was expected. A comparison of the frequencies of a *truei* male "out" with the frequencies of a *comache* male "out" revealed that *truei* was significantly out more often than *comanche* (chi-square = 10.1). Females were recorded out of the aggregation 9.0% of the time. However, the expected probability was exceeded by the observed frequencies only in the number of times that the *comanche* female was with two males while the other female nested alone.

In these tests the number of pairings and cross-pairings was low. The double pairs observed (2.9%) were less than the expected (4.7%), whereas the double cross-pairs fell very close to the expected percentage. The difference between the records of double pairs and double cross-pairs is not significant, nor is that between the simple pairs and simple cross-pairs. From these data it can be said that the mice do not demonstrate a strong tendency to associate only with others of their kind. This is shown particularly when the total pairings are compared with the total cross-pairings (chi-square = 3.0). These were not significant deviations.

Thirteen experiments resulted in wounds or death to males. In seven experiments a male was killed, and five of these were males of the species *truei*. Non-fatal wounds were evidenced by the *comanche* male in only one experiment, whereas five resulted in wounds to the *truei* male. In these tests male rivalry was pronounced. The *comanche* male, however, was more aggressive and succeeded in nesting with the two females more often than did the rival *truei* male.

nasutus with *truei*

In tests between these species the expected frequency was exceeded in five of the 15 possible categories (table 1). The combination of four mice in one compartment occurred a very highly significant number of times. This aggregation com-

TABLE 2. *Summary of data from intraspecific and interspecific tests between* Peromyscus comanche, P. ..asutus, *and* P. truei

	comanche with *comanche*	*nasutus* with *nasutus*	*truei* with *truei*	Totals	Percentage	*comanche* with *nasutus*	*comanche* with *truei*	*nasutus* with *truei*	Totals	Percentage
Total observations	422	525	488	1435	—	567	435	550	1552	—
All together	65	242	203	510	35.5	250	143	231	624	40.2
Single pairs	23	8	6	37	2.6	15	9	22	46	3.0
Double pairs	22	12	8	42	2.9	17	13	19	49	3.1
All pairs	45	20	14	79	5.5	32	22	41	95	6.1
Single cross-pairs	26	15	18	59	4.1	21	15	18	54	3.5
Double cross-pairs	23	15	11	49	3.4	10	20	11	41	2.6
All cross-pairs	49	30	29	108	7.5	31	35	29	95	6.1
Number of males killed	6	2	4	12	—	0	7	0	7	—
Number of males wounded	9	2	7	18	—	4	6	2	12	—

prised 42.0% of the 550 observations, and was the most frequently observed combination. The other four categories which exceeded the expected probability were when a male or a female nested separately from the aggregation. A *nasutus* male was out of the aggregation in 12.7% of the observations, and a *truei* male was "out" in 12.2%. A female nested separately from the aggregation 14.2% of the observations. Although no statistical significance was detected between the frequencies that individuals of the same sex separated from the aggregation, a comparison of the times that individuals of different sexes nested separately indicated that the data were not due to chance (chi-square = 16.2).

The number of observed double pairs did not differ significantly from the number expected. Moreover, other categories of pairing and cross-pairing were considerably less than expected. A comparison of the interspecific and intraspecific single pairings and cross-pairings resulted in data of no statistical significance, nor did a comparison of double pairs and double cross-pairs. Likewise no significant difference was seen between all pairings and all cross-pairings (chi-square = 2.1).

No individuals were killed in these series of tests, and no wounds were evidenced by *truei* mice. On three occasions a different *nasutus* male was wounded. Physical manifestations of male dominance were less in these interspecific tests than in the two other series.

INTERPRETATIONS AND DISCUSSION

Several important types of information were gained from these tests, and a summary of the data is presented in table 2. Although there appears to be good agreement in the behavior of the three species, certain social arrangements were more frequently observed than others. Pairing frequencies were lower in the interspecific tests than in the intraspecific tests, and only 6.1% of the observations were of this nature. Cross-pairings were as frequent as pairings, and 12.2% of the 1,552 observations were of these categories. Moreover, quartet aggregations showed the highest frequency of all the combinations (40.2%), and male dominance was pronounced.

Nevertheless there are some differences between these species when they are tested together. The proportion of *nasutus* males wounded or killed was low, indicating a higher survival value in these tests than that of the other males. Although males of each species were excluded from the aggregation an equal amount of time, females did not behave similarly. *P. comanche* females were out more frequently when tested

with *nasutus* than with *truei*. Likewise the percentage of *truei* females nesting separately was higher when paired with *nasutus* than with *comanche*. Moreover, *nasutus* females were in the nesting group more often than females of the other two species. In general the behavioral stability of *nasutus* in the interspecific tests was greater than the other sibling species. Pairing frequencies were significantly highest when *truei* and *nasutus* were tested together and lowest with combinations of *comanche* and *truei*.

There seems to be little difference between the intraspecific and the interspecific tests in social pattern. The percentage of cross-pairings (7.5), however, was higher than the percentage of pairings (5.5) in the intraspecific tests. Table 2 shows that in the intraspecific tests *comanche* paired and cross-paired most frequently, and totals of these categories were significantly greater than for *nasutus* or *truei*. Moreover, remarkably few quartet aggregations were formed by *comanche*. This species showed the highest rate of pairing by sexes, and arrangements comprising any two individuals were preferred to other combinations. With *comanche* the percentage of pair formations by members of the same or different sexes was 44.8.

In the intraspecific tests the degree of male dominance varied, but the percentages of males "out" are very similar for *comanche* and *truei*. Although not significantly greater than *truei*, the results with *nasutus* were significantly different from *comanche*. *P. nasutus* males were seldom wounded or killed, and the few manifestations of physical dominance and the high number of quartet aggregations indicate the compatibility of *nasutus* males in the intraspecific situation.

The inference from these data is that the species of the *truei* group do not pair significantly with their own kind and therefore do not discriminate between their own in intraspecific tests and alien species in interspecific tests. These experiments were performed with the view of determining from the social pattern the degree of preference, but the results imply that interspecific isolating mechanisms are lacking in this group on the behavioral level, at least when exposed to each other in the test situation.

There is no completely satisfactory explanation for the failure of these mice to discriminate, but the low frequency of pairing in interspecific tests is not exceptional when male dominance and gregariousness appear to be the rule. Both of these behavior patterns seem to be the most predictable, and if present in the social organization, they would effectively block any pairing reactions of the mice.

The presence of interspecific isolating factors have been demonstrated in several groups of the genus *Peromyscus*. The existence of social isolation has been clearly shown in *P. maniculatus* and *P. polionotus* of the *maniculatus* group (Blair and Howard, 1944); in *P. pectoralis* and *P. boylei* of the *boylei* group (Clark, 1952); and *P. leucopus* and *P. gossypinus* of the *leucopus* group (McCarley, 1953; Bradshaw, 1956). In each of the series of tests conducted by these workers the incidence of pairing by species was sufficiently high to be considered as definite evidence of social isolation in mixed societies. These species of *Peromyscus* discriminated against other kinds and showed a significant preference for their own.

The results of Blair (1953) with *P. nasutus* and *P. truei* are consistent with those reported above. In his experiments the low frequency of cross-pairing and the high incidence of pairing were taken by Blair as an indication of discrimination between members of the same and the other population. The frequency of single and double pairings was outstanding. The totaled pairing scores accounted for 73.3% of his observations, whereas the three classes of cross-pair scores together comprised only 5.6%. In the present study none of the 20 experiments pairing *nasutus* with *truei* resulted in high pairing scores. The maximum number of pairings by individuals of the same species was six. The frequency of cross-pairing, although less than that of pairing, was con-

siderably more by ratio when compared with the low incidence reported by Blair. The cause of the disparity between the results of the two different sets of tests in unknown. No intraspecific experiments were conducted by Blair, and his three experiments may have represented too small a sample from which to draw statistical conclusions.

Male dominance was reported by these workers in many tests, and was frequently accompanied by fighting between males. However, indications of male aggressiveness were most evident in tests of pure societies and almost absent in mixed species tests. In the *P. maniculatus, boylei,* and *leucopus* species groups, polygamy does not seem to be a normal social arrangement unless more than one female of the same species is present.

Summary

Three species of white-footed mice (*Peromyscus comanche, P. nasutus,* and *P. truei*) were tested in a four-compartment cage to determine from the social patterns the degree of preference in intraspecific and interspecific groupings. Combinations expressing gregariousness and male dominance were the most common social interactions. Social combinations involving the pairing of a male and a female of the same or different species occurred less frequently than would be expected with random distribution of the mice in the compartments. Pairing frequencies were low in the interspecific tests, and in these cross-pairings between species were as frequent as pairings by species. Although the pairing frequencies were significantly higher when *nasutus* and *truei* were tested together, the inference from the data of all tests is that the species of the *truei* group do not discrimminate between their own in intraspecific tests and alien species in interspecific tests. The results gave no clear evidence of interspecific isolating mechanisms at the social level in this group.

Acknowledgments

I wish to express my appreciation to W. Frank Blair for suggesting this study and for valuable advice throughout the course of the project. Alice Louise Baartz and John G. Riemann assisted in many aspects of the laboratory work, and T. E. Kennerly read the manuscript. The Laboratory of Vertebrate Speciation, University of Texas, partially supported the study.

Literature Cited

Blair, W. F. 1953. Experimental evidence of species discrimination in the sympatric species, *Peromyscus truei* and *P. nasutus*. Amer. Nat., **87**: 103–105.

——. 1954. Tests for discrimination between four subspecies of deer-mice (*Peromyscus maniculatus*). Tex. J. Sci., **6**: 201–210.

——. and W. E. Howard. 1944. Experimental evidence of social isolation between three forms of mice of the cenospecies *Peromyscus maniculatus*. Contr. Lab. Vert. Biol., Univ. Mich., **26**: 1–19.

Bradshaw, W. N. 1956. Species discrimination in the *Peromyscus leucopus* species group of mice. Unpubl. M. A. thesis, Univ. Texas, 53 p.

Clark, W. K. 1952. Isolating mechanisms, competition, and geographic variation of the *Peromyscus boylei* group in Oklahoma and Texas. Unpubl. Ph. D. dissertation, Univ. Texas, 102 p.

Dice, L. R. 1929. A new laboratory cage for small mammals, with notes on methods of rearing *Peromyscus*. J. Mamm., **10**: 116–124.

——. 1940. Speciation in *Peromyscus*. Amer. Nat., **74**: 289–298.

McCarley, W. H. 1953. Biological relationships of the *Peromyscus leucopus* species group of mice in eastern Texas. Unpubl. Ph. D. dissertation, Univ. Texas, 85 p.

Mayr, Ernst. 1949. Systematics and the origin of species. Columbia Univ. Press, N. Y.

Svihla, A. 1932. A comparative life history study of the mice of the genus *Peromyscus*. Misc. Publ. Mus. Zool., Univ. Mich., **24**: 1–39.

NOTES AND COMMENTS

A POSSIBLE PHYLOGENETIC INTERPRETATION OF THE ANNELIDICOLOUS COPEPOD *PHYLLOCOLA PETITI* DELAMARE & LAUBIER

R. V. Gotto
Department of Zoology, Queen's University, Belfast

The discovery by Delamare-Deboutteville and Laubier (1960a) of an extremely aberrant copepod infesting phyllodocid worms raises anew the phylogenetic questions invariably posed by highly specialized parasitic Crustacea. To recapitulate briefly the known facts in this particular case, the French authors found three specimens in varying stages of development on *Eulalia pusilla* Oersted and *Phyllodoce* sp. from the Mediterranean coast of France. The parasites have a small bulbous body (about 0.5 mm long in the adult female), and are fixed externally to the skin of the host, this attachment being effected anteriorly by a short siphon from which extend two long absorptive horns within the body of the worm. These latter ramify among the host's parapodial muscles. Short, unjointed spinous antennules are present, as well as two pairs of appendages which are thought to represent the antennae and maxillipeds. Perhaps the most remarkable feature, however, are the egg-masses. These take the form of two slender filaments made up of the conjoined peduncles of individual ova. There is thus no egg-sac in the accepted sense of the term. After reviewing the various aberrant copepods which parasitize annelids. (*Xenocoeloma, Aphanodomus, Flabellicola*, the herpyllobiids and the monstrillids), the discoverers of *Phyllocola petiti* conclude that its affinities do not lie with any of these forms (Deboutteville and Laubier, 1960b).

Now clearly Deboutteville and Laubier can best evaluate the relationships of this bizarre parasite. It is, nevertheless, tempting to speculate on the phylogeny of so curious a copepod. In this connection, it might be revelant to consider the possibility that *P. petiti* may be derived neotenously from monstrillid or monstrillid-like ancestors.

It is well known that the Monstrillidae exhibit protelean parasitism, their nauplii burrowing into serpulids, syllids, and spionids, or even into prosobranch molluscs. Once installed in a suitable host, the nauplius is nourished through the medium of two (sometimes one or three) long absorptive processes, prior to a metamorphosis which ultimately results in a free-living and strongly swimming adult lacking not only mouthparts but an alimentary canal as well. Although mentioning the resemblance between these nutritive filaments of monstrillid larvae and those of the adult *Phyllocola*, the French writers are inclined to regard this as a case of pure convergence.

Obviously too much importance should not be placed on the presence in both groups of two such similar structures as these absorptive horns. Nutritional "pipelines" of comparable type are, after all, fairly widespread among specialized crustacean parasites which are clearly unrelated. It is nevertheless worthwhile to examine this question carefully, on the chance that something more than mere convergence may be involved. To suggest a neotenous origin for the Phyllocolidae is perhaps not so far-fetched as it seems, when the following facts are taken into consideration:

1. It appears to be fairly generally accepted that neoteny has been operative in copepod evolution at various times, on various levels, and in various ways (see, e.g., de Beer, 1930 and 1958; Gurney, 1942; Margalef, 1949; Rose and Vaissière, 1952; Serban, 1960). To invoke it as a possible mechanism in the present instance is not, therefore, an especially revolutionary concept.
2. The monstrillids, although apparently associated mainly with serpulids, are not confined to this family—or even to the phylum Annelida. It would not be particularly surprising if certain monstrillid species had, in the remote past, utilized phyllodocids as hosts.
3. The reduction of mouthparts in the adults of both families, although certainly capable of alternative explanation, at least does not disqualify the hypothesis advanced here.
4. The occurrence of similar absorptive filaments, although likewise possibly attributable to convergence, could also, on the present view, be considered as a neotenous retention in the Phyllocolidae.
5. The very curious manner in which the ova are attached to the female *Phyllocola* is an extremely striking feature. But the Monstrillidae are also distinctly aberrant in their method of egg attachment. In this family the ova are produced in a single elongate mass and are agglutinated not only to each other but to two long and slender filaments issuing from the ventral surface of the genital segment. These fine bristles thus form a central supporting axis for the egg mass, and in some species (e.g., *Cymbasoma longispinosum* Bourne) may be fully as long as the entire body. Once more, therefore, no true egg-sac is formed. Can it be that here again we are confronted with a fundamental, though transmuted, pattern, rather than a shadowy and fortuitous resemblance?
6. Finally, a comparison of the *Phyllocola* antennule with that belonging to several monstrillid

species is not without interest in the present context. The former is rather short and unjointed, though its derivation from an archetype consisting of a small number of segments can easily be envisaged. It is fairly thickly adorned with short spinous setae, as well as a single large claw-like protuberance. The significant point of interest lies in the fact that a number of monstrillids not only posses a stout antennule often provided with short spine-like setae, but that in at least three species the segmentation is almost completely obliterated. While coalescence of a few antennular segments is of fairly frequent occurrence within the Copepoda, it is distinctly rare to find practically the whole antennule thus involved. In short, comparatively little modification of this appendage would be necessary to produce the type of antennule seen in *P. petiti*.

To sum up, then, it may not be wholly unreasonable to put forward the following tentative hypothesis: The Phyllocolidae may be descended from monstrillid or monstrillid-like copepods which, in previous times, passed their larval stages in phyllodocid worms just as their modern representatives do in other polychaetes. Within this ancestral line (and perhaps abetted by a lengthening larval period) neoteny supervened prior to final metamorphosis with its detailed build-up of adult characters. The absorptive horns would be retained, though somewhat modified, and would remain within the host as a matter of nutritional necessity. The rest of the female's body, however, would come to project through the worm's bodywall, in order to facilitate reproduction and the release of nauplii. This latter step might entail a strengthening of the slender connection between the internal and external portions of the parasite, and result in the elaborate chitinization of this region described by Debouttéville and Laubier. The extrusion of ova which are not enclosed within an egg-sac could be regarded as an evolutionary legacy inherited from monstrillid ancestors, suitably adapted to the copepod's present ecological requirements. The life cycle of the male would probably be somewhat different and could well have involved the retention of a free-swimming period as an adult. (Since no certain male has yet been found, *Phyllocola* may even prove to be hermaphroditic.) Later generations, in the course of time, might have evolved more and more in the direction of external parasitism, the young infective stages settling on the skin of the host which would be pierced by the development of the siphon from which arise the nutritive processes.

In conclusion, it cannot be sufficiently emphasized that the reconstruction attempted here is for obvious reasons entirely speculative—difficulties and objections are clearly legion. In particular it may be considered (with a good deal of justification) that to invoke wholesale neotenous phenomena is a dubious procedure—so many opportunities are thereby presented for theorizing ahead of one's data. It is certainly true that many a zoological problem-child has, within recent years, been left on neoteny's doorstep in the hope that there, perhaps, resides the answer to its parentage. But it may be that out of a discussion of such possibilities the truth will eventually emerge.

Literature Cited

Beer, G. R. de. 1930 Embryology and Evolution. Oxford.

——. 1958. Embryos and Ancestors. 3rd Edition. Monographs on Animal Biology.

Delamare-Debouttéville, C., and L. Laubier 1960a. Les "Phyllocolidae," une famille nouvelle de copépodes parasites d'annélides polychètes. C. R. Acad. Sci. Paris, **251**: 2083.

——and——. 1960b. Les "Phyllocolidae" Delamare et Laubier, copépodes parasites d'annélides polychètes phyllodocides, et leurs rapports avec les copépodes annélidicoles. C. R. Acad. Sci. Paris, **251**: 2231.

Gurney, R. 1942. Larvae of Decapod Crustacea. Ray Society, London.

Margalef, R. 1949. Importancia de la neotenia en la evolucion de los Crustaceos de aqua dulce. Publ. Inst. Biol. Aplic. Barcelona, **6**.

Rose, M., and R. Vaissière. 1952. *Corina granulosa* Giesbrecht, 1892 et *Corina longifurca nouvelle espèce*. Ann. Sci. Nat., Zool. (11): 14.

Serban, M. 1960. La néotenie et le problème de la taille chez les copépodes. Crustaceana, **1**: 77.

LARVAL COMPETITION AND THE ORIGIN OF TETRAPODS

FREDERICK E. WARBURTON AND NORRIS S. DENMAN
6171 Sherbrooke St., W., Montreal 28, Quebec, and
c/o Aluminum Company of Canada, Ltd., Montreal 3, Quebec

There has been a recent flurry of speculation about the ecological situation leading to the evolution of tetrapods. The basis for discussion is Romer's (1933, 1945) hypothesis that an arid environment conferred a selective advantage on lunged fishes capable of escaping from drying ponds and streams by crawling over land. Three authors in particular, Orton (1955), Gunter (1956), and Inger (1957), have proposed alternative hypotheses. Their objections to Romer's hypothesis are: first, in the severe aridity postulated by Romer, water might be too scarce to maintain a breeding population, while a wetter environment would fail to exert the necessary selection. Secondly, Gunter suggests that Romer's hypothesis involves "evolution without selection," as the lobe-fin-*cum*-limb has no advantage in its intermediate stages. Thirdly (though this is not explicitly stated), there is the probability of competitive exclusion of the proto-amphibian by the lunged fishes; presumably the proto-amphibian spent most of its life living in water in competition with fish which, as fish, would *a priori* be more efficient, and cause a Gausean extermination of the proto-amphibian.

One of us (N.S.D.) is preparing a fourth alternative to Romer's hypothesis. While arguing the pros and cons of this new hypothesis, we envisioned a situation which would save the important features of Romer's theory while avoiding the Scylla of dessication and the Charybdis of competitive exclusion, and yet give the limb survival value at every stage of its evolution from the fin.

Assume that, as in most modern amphibians and fishes, the young of proto-amphibians and the contemporary lunged fresh-water fishes were far more numerous than the adults. Even today, larval salamanders and larval lungfish are remarkably similar morphologically. Therefore, competition might have been intense between the larvae of lunged fishes and proto-amphibians, though the relatively scarce adults could live together in comparative harmony. Both forms could co-exist in the same region *provided their larvae were ecologically separated.* If, as Romer supposes, the Devonian was a time of seasonal floods alternating with drought, there is a simple way to separate the two larval populations: have the proto-amphibian lay its eggs in the flood pools. The more conservative fishes perhaps laid their eggs in deep holes, under sunken logs, and in weed beds; the earliest proto-amphibians differed only slightly, and only in a behavioral way, by laying their eggs in the shallowest water they could swim to during the floods.

There would come a time annually when the pools dried up enough to force the young proto-amphibians to migrate back to permanent water, perhaps stimulated by crowding and light evening showers, as suggested by Ewer (1955) and the Goins (1956). The breeding pools might then be separated from permanent water by shallows, or by a fraction of an inch to many yards of land, so *any degree of "walking" ability would be advantageous and selected for,* from a grunnion-like flop to a nimble scurry. Furthermore, selection would be intense, and would operate on a large number of larvae, rather than on the small adult population, probably already randomly decimated, postulated by Romer.

One must be a successful tadpole before one can be a successful frog, and great evolutionary advances are just as likely to be adaptations for larval survival as for adult survival. It seems plausible that the proto-amphibian was able to survive and increase, even though its larva was no better than a fish larva, and the adult perhaps not quite as good as an adult fish, solely because its metamorphosis into a crude sort of tetrapod allowed it to survive one brief but critical excursion across a few inches of mud, and let it utilize extensive breeding grounds which were otherwise unattainable.

LITERATURE CITED

EWER, D. W. 1955. Tetrapod limb. Science, **122**: 467–468.

GOIN, C. J., AND O. B. GOIN. 1956 Further comments on the origin of tetrapods. EVOLUTION, **10**: 440–441.

GUNTER, G. 1956. Origin of the tetrapod limb. Science, **123**: 495–496.

INGER, R. F. 1957. Ecological aspects of the origin of tetrapods. EVOLUTION, **11**: 373–376.

ORTON, G. L. 1954. Original adaptive significance of the tetrapod limb. Science, **120**: 1042–1043.

ROMER, A. S. 1933. Vertebrate Paleontology. Univ. Chicago Press, Chicago.

——. 1945. *Ibid.* Second edition.

STUDIES IN WARNING COLORATION AND MIMICRY. III. EVOLUTION OF MÜLLERIAN MIMICRY

JAMES E. HUHEEY
Worcester Polytechnic Institute, Worcester, Massachusetts

For convenience, defensive mimicry is often classified as either Batesian or Müllerian, depending upon whether the relationship is between a model and one or more mimics, or between two or more models. These two types of mimicry have been discussed thoroughly elsewhere (cf. Fisher, 1958; Sheppard, 1958, 1959) and need not be reviewed here. Let it suffice to point out that the two categories are not mutally exclusive, but merely limiting cases of a presumably complete spectrum of possibilities with the mimic ranging in undesirability from being completely innocuous on the one hand, to being fully as potent as its model, on the other.

Discussions of Müllerian mimicry generally point out the advantages to be gained by two or more models when using the same (or similar) aposematic signals and thus reducing losses from predation as predators learn the signal by random sampling. Unfortunately, the impression left with the reader is that evolution of Müllerian mimicry proceeds through convergence of color pattern of two previously developed models. While this may be the case in some, perhaps even the majority of cases, another evolutionary pathway also seems possible.

The most critical period in the evolution of unpalatability in prey is during the early stages when such unpalatability may not be widespread in the population. This problem has been discussed in some detail by Fisher (1958). While the difficulties involved probably are not of such magnitude that unpalatability cannot arise through simple selection, it must be admitted that it seems likely that unpalatable characteristics are often eliminated from populations when in the incipient stages simply because the bearers did not survive the tasting process, and the characteristic was not as yet sufficiently widespread to benefit the entire population. This is true even if, as seems probable, such characteristics arise by a gradual accumulation of favorable mutations rather than arising full-blown in a single mutation.

It has been shown by recent experimental work (Brower, 1958a, b, c, 1960) that after being exposed to models, predators still occasionally sampled models and mimics. However, the predators seemed to be inhibited by previous experiences with models. For example, sometimes even mimics which proved to be completely palatable to control birds were apparently "tasted" and yet rejected by experienced birds. A predator so conditioned would be expected to attack an individual exhibiting a known aposematic signal more cautiously and respond to an unpleasant stimulus more readily than one not so conditioned. Under these circumstances, the prey would undoubtedly have a better chance of surviving the sampling experience.

It can be seen, therefore, that a Batesian mimic is ideally situated with respect to the development of protective distastefulness. Being protected from vigorous attack by the model, any tendency to develop distasteful qualities will be enhanced.

It may be argued that since a Batesian mimic is already protected by its mimicry, the selective advantage of developing distastefulness will not be sufficiently great to cause it to become established. However, some mimics are always lost as predators learn or make mistakes. Even if this were not the case, mimics belonging to polymorphic species would be more susceptible to such pressures. Presumably in these species polymorphism occurs because the model is not sufficiently abundant to protect the entire population if it were completely of the mimetic form (Fisher, 1958; Sheppard, 1958). The numbers of mimetic and non-mimetic individuals in such a population are adjusted by selection so that both forms receive equal protection. Thus, the frequency of mimics and the protection received by these mimics is severely limited by the frequency of the models. Any mechanism which frees the population from such control will be of selective advantage.

The mechanism of development of Müllerian mimicry advanced here might be capable of confirmation by field studies in an area rich in mimetic forms. Given two closely related allopatric species or subspecies of models, A and A′, one of which can be shown to have a Müllerian mimic B, the divergence of color pattern between A and A′ will be an indication of the mode of formation of the Müllerian relationship. If A and A′ are widely different in their aposematic signal, the chances are that A and B have developed their patterns through convergent evolution of both forms. If, on the other hand, A and A′ do not differ appreciably, the chances are that B originally was a Bastesian mimic of A, since, as Fisher (1958) and Sheppard (1958) have pointed out, development of Müllerian mimicry between two species of models causes them to compromise their signals rather than one species changing to conform to the other. [1]

[1] In addition, a reviewer has suggested that the presence of a single Müllerian model in a taxon otherwise consisting only of innocuous forms would be good support for the present hypothesis.

LITERATURE CITED

BROWER, J. VZ. 1958a. Experimental studies of mimicry in some North American butterflies. I. *Danaus plexippus* and *Limenitis archippus archippus*. EVOLUTION, **12**: 32–47.

——. 1958b. Experimental studies of mimicry in some North American butterflies. II. *Battus philenor* and *Papilio troilus, P. polyxenes,* and *P. glaucus*. EVOLUTION, **12**: 123–136.

——. 1958c. Experimental studies of mimicry in some North American butterflies. III. *Danaus gilippus berenice* and *Limenitis archippus floridensis*. EVOLUTION, **12**: 273–285.

——. 1960. Experimental studies in mimicry. IV. The reactions of starlings to different proportions of models and mimics. Amer. Nat., 94: 271–282.

FISHER, R. A. 1958. The genetical theory of natural selection. Second revised edition, Dover, New York, 291 p.

SHEPPARD, P. M. 1958. Natural selection and heredity, Hutchinson, London, 212 p.

——. 1959. The evolution of mimicry; a problem in ecology and genetics. Cold Spring Harbor Symp. Quant. Biol., **24**: 131–140.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Allan, H. H. 1961. Flora of New Zealand. vol. I. Indigenous Tracheophyta. LIV + 1085 pp. illus. 5 gns. Government Printer, Lambton Quay, Wellington, N. Z. A comprehensive description of indigenous ferns, conifers, and dicotyledonous flowering plants. Includes an introductory essay, a bibliography, glossary, list of Maori names of plants, and other supplementary material.

Banton, M. (ed.). 1961. Darwinism and the Study of Society. XX + 191 pp. 5 figs. $5.00. Travistock Publications (London) and Quadrangle Books (Chicago). Proceedings of centenary symposium held at the University of Edinburgh, with an introduction by J. Bronowski and papers by social scientists and biologists. The latter includes contributions by Lancelot Hogben (Darwinism and Human Society in Retrospect), C. H. Waddington (The Human Evolutionary System), J. Maynard Smith (Evolution and History), and S. A. Barnett (Communication in Animal and Human Societies).

Sager, Ruth and Francis J. Ryan. 1961. Cell Heredity. XI × 411 pp. illus. $7.50. John Wiley. A textbook of genetics dealing only with the molecular and cellular level. The subject of evolution is not considered.

Corliss, John O. 1961. The Ciliated Protozoa: Characterization, classification, and guide to the literature. 310 pp. 22 plates. $12.00. Pergamon Press. Oxford. A monograph on the systematics of the subphylum Ciliophora covering some 1400 supraspecific names, and including a reference list of 1700 titles.

Colbert, Edwin H. 1961. Dinosaurs. Their Discovery and their World. XIV + 300 pp. 151 illus. $7.50. Dutton. N.Y. An account for the general reader of the discovery of dinosaurs, methods of studying them, their evolutionary history from origin to extinction, their systematics. Study aids include a brief directory of museum exhibits and a selected bibliography of books. Two-thirds of the illustrations represent glossy plates of high quality; the remainder are line drawings. The author is the outstanding authority on the subject and a former editor of this journal.

Blair, W. Frank (ed.). 1961. Vertebrate Speciation. XVI + 642 pp. illus. $8.50. University of Texas Press, Austin. Proceedings of a symposium held in 1958 at the University of Texas. The 21 papers and transcript of discussion are included, organized in five sections: 1) isolating mechanisms; 2) evolution of behavior; 3) polymorphism and polytypic species; 4) population dynamics; 5) age and origin of species.

Swinton, W. E. 1961. The Wonderful World of Prehistoric Animals. 93 pp. illus. Price not stated. Doubleday. N. Y. A lavishly illustrated book on paleontology for juvenile readers.

ANNOUNCEMENT

EVOLUTION is now available on microfilm. Beginning with vol. 16 (1962), microfilm of current issues will be sold only to bona fide subscribers to the journal. Copies of earlier numbers may be purchased by anyone. Copies of individual articles cannot be purchased. Address all orders to: University Microfilms, Inc., 313 N. First St., Ann Arbor, Michigan.

SOCIETY FOR THE STUDY OF EVOLUTION

MINUTES OF THE SIXTEENTH ANNUAL BUSINESS MEETING

The meeting was called to order at Purdue University, West LaFayette, Indiana, at 9:10 AM, August 30, 1961, with President Harlan Lewis in the chair.

1. The minutes of the fifteenth Business Meeting, held at New York, New York, December 29, 1960, stood approved as published.
2. The Secretary reported on the interim activities of the Council. During the year the Council acted on the Constitutional amendments which were later submitted to the membership for balloting. Joint symposia were arranged for the present meeting and President Lewis made the following appointments: Dr. Grady Webster as the Society's local representative for the AIBS meetings at Purdue, Dr. Webster and Dr. C. B. Heiser to plan the joint symposium with the American Society of Plant Taxonomists, and Dr. Leland Chandler and Dr. T. E. Moore to plan the joint symposium with the Entomological Society of America.
3. The following officers and Councillors were elected for 1962: *President-Elect*, John A. Moore; *Vice Presidents*, I. Michael Lerner, Antonio Brito da Cunha, N. Tinbergen; *Secretary*, Herbert H. Ross; *Editor*, C. D. Michener; *Councillors*, James F. Crow, O. H. Frankel.
4. The above elections left vacant the two councillors of the Class of 1962. On behalf of the Nominating Committee, Michael J. D. White and Everett Olson were nominated for these two vacancies. After a call for further nominations it was moved and seconded that the nominations be closed and the Secretary cast a unanimous ballot for these nominees. Motion carried.
5. Reports of the retiring Editor, the Managing Editor, and the Treasurer were read and discussed.

   It was moved and seconded that an expression of our deep appreciation for his unflagging and highly efficient efforts, and his great service to the Society, be conveyed to the retiring Editor. Motion carried by acclamation.
6. The following summary was presented of action taken at the preceding Council Meeting.

   a. The following Associate Editors for EVOLUTION were appointed: R. D. Alexander (U.S.A.), D. I. Axelrod (U.S.A.), A. J. Cain (Great Britian), E. Goldschmidt (Israel), C. B. Heiser (U.S.A.), M. J. Heuts (Belgium), E. P. Odum (U.S.A.).

   b. Because of the increase in manuscripts being submitted to EVOLUTION and because the Society's finances seem to be on a little firmer ground than they were two years ago, $14,000 was approved as the 1962 budget for printing EVOLUTION.

   c. Dr. Wilma Lehmann was appointed as the Indexing Committee, and is to be given such help as the Managing Editor suggests.

   d. Dr. T. E. Moore was appointed as a committee of one to solicit advertising for EVOLUTION.

   e. A request for funds to assist with financing the XVI International Congress of Zoologists, to be held August 21–27, 1963, in Washington, D. C., was discussed. The Secretary was instructed to write the Secretary-General of the Congress to see if it would be possible for the Society to solicit voluntary contributions to be used to defray the expenses for travel of overseas scientists to attend the Congress. If the Congress were willing to accept monies for these purposes, the Treasurer was instructed to add an appropriate line to the next general billing of the Society's members.
7. The 1962 meeting will be held in con-

junction with the AIBS at Corvallis, Oregon, August 26–30.

8. The meeting adopted an expression of appreciation to the AIBS administration, to Purdue University, to our local representative, and to those who assisted in planning the program of the meeting for their great assistance and many courtesies which have made this annual meeting of the Society highly successful.

INDEX TO VOLUME XV, 1961

Adaptation, evolution of, in termites, 117
Algae, soil; ecological niche relationships, 228
Alston, R. E., 352
Anolis, thermal relations, 98
Antibodies, in cattle, 196; in Columbidae, 31
Antigens, cellular, in Columbidae, 30; in American buffalo and domestic cattle, 196; in bullfrog tadpoles, 267
Aspects, ecological, of flower evolution, 44
Bacteriophage, conditions for existence, 153; general properties, 153; population dynamics, 157
Baker, H. G., 378
Barrier, East Pacific and marine shore fishes, 545
Beetles, lycid, mimetic assemblages, 15
Birch, L. C., 360
Birds, variation in wing length and body size, 180
Bison bison, taxonomic status, blood groups, 196
Blair, W. F., 352
Bos taurus, taxonomic status, blood groups, 196
Bowman, R. I., 407
Briggs, J. C., 545
Brncic, D., 92
Buddleia, polyploidy, phylogeny, photoperiodism, 272
Bufo, secretory substances of paratoid glands, 352
Bullfrog populations, histocompatibility, 267
Campbell, A., 153
Carson, H. L., 496
Chang, W-T., 326; 418
Characters, vestigial, of termites, 115
Christiansen, K., 288
Chromosome divergence in *Tripsacum* and *Zea*, 394
Chromosome fitness changes, in *Drosophila persimilis*, 340
Chromosome interchange in rye, consequences of, 145
Chromosome polymorphism in *Drosophila persimilis*, 535
Chromosome types in rye, survival of, 145
Classes, zoophilous, flower, 44
Clatworthy, J. N., 209
Clifford, H. T., 455
Cohabitation of closely related species, 222
Collembola of caves: convergence and parallelism, 288
Color, aposematic, in *Dasypeltis*, 72; in lycid beetles, 15
Color, mimetic, in lycid beetles, 15
Color pattern, variation in *Dasypeltis*, 77
Color, procryptic, in *Dasypeltis*, 72
Color variant in *Plethodon cinereus*, 281
Columbidae, cellular antigens, 30
Competition, isoallelic, in *Drosophila melanogaster*, 239
Competition, larval and tetrapod origin, 566
Cook, L. M., 478
Copepod, *Phyllocola petiti*, possible phylogeny, 564
Corn and *Tripsacum* chromosome divergence, 394
Crassulaceae, cytotaxonomic problems, 375
Cytotaxonomy, some problems, in Crassulaceae, 375
Dacus, natural selection between two species, 360
Daly, K., 60
Dasypeltis, mimicry, 72
del Solar O., E., 401
Dendroica kirtlandii, breeding habitat; cowbird parasitism, 174
Denman, N. S., 566
Dobzhansky, T., 263 (Book review); 461
Donald, H. P. (Book review), 380
Drosophila ananassae, peculiar gene arrangements, 486
Drosophila gaucha, courtship and sexual isolation, 401
Drosophila melanogaster, heat resistance, geographical variation, 1; heterosis; fitness, 496; isoallelic competition, 239
Drosophila pavani, courtship and sexual isolation, 401; integration of genotype, 92
Drosophila persimilis, chromosomal fitness changes, 340; mating propensity of homokaryotypes, 535
Drosophila pseudoobscura, developmental homeostasis, 132; frequencies and allelism of lethal factors, 447
Drosophila serrata, evolutionary status, 461
Drosophila simulans, heat resistance, geographical variation, 1
Dunn, L. C., 385
Ecological niche of soil algae, 228
Ecotypic differentiation, 166
Edibility of lycid beetles, 23
Eisner, T., 15
Emerson, A. E., 115
Epling, C., 447
Eumenidae, Indo-Australian, evolution, 468
Evolution, floral, in Gramineae, 455
Evolution, flower, ecological aspects, 44
Evolution in Indo-Australian wasps, 468
Evolution of Müllerian mimicry, 567
Evolution, progressive, correlated with regressive, 118
Evolution in pseudogamous *Ranunculus auricomus*, 510
Evolution, regressive in termites, 115
Feeding specialization of moth *Panaxia*, 478
Fishes, marine shore, distribution, 545
Fishes, *Poeciliopsis*, reproductive mechanism, 302
Fishes, trans-Pacific shore: list, 552
Freire-Maia, N., 486
Gamodemes, *Plantago lanceolata*, leaf length responses, 166
Gans, C., 72
Gene arrangements in *Drosophila ananassae*, 486
Genetic drift in *Rattus rattus*, 259

Gotto, R. V., 564
Gramineae floral evolution, 455
Gregor, J. W., 166
Grüneburg, H., 259
Haas, R., 267
Habitat segregation in *Anolis*, 99
Hadorn, E., Developmental genetics and lethal factors (H. P. Donald), 380
Hamilton, T. H., 180
Homokaryotypes, mating propensity, *Drosophila persimilis*, 535
Harper, J. L., 209
Heat resistance in *Drosophila*, 1
Heiser, C. B., Jr., 247
Helianthus annuus, hybrids, changes in variation pattern, 60
Helianthus Bolanderi, hybrids, changes in variation pattern, 60
Helianthus petiolaris, morphological and cytological variation, 247
Heterosis and fitness in *Drosophila melanogaster*, 496
Hildemann, W. H., 267
Hochman, B., 239
Homeostasis, developmental, in *Drosophila pseudoobscura*, 132
Homeostasis in termites, 118
House mice, dynamics of a variant *t*-allele, 385
Huheey, J. E., 567
Hunsaker, II, D., 352
Interchange in chromosomes of rye, 145
Inversions in chromosomes of *Drosophila pavani*, 92
Irwin, M. R., 30
Kirtland's warbler, cowbird parasitism; population, 174
Klots, A. B., 15
Koref-Santibañez, S., 401
Lampreys, European: life-history, systematics, distribution, neoteny, speciation, 523
Langer, B., 535
Lethal factors within and between gene arrangements in *Drosophila pseudoobscura*, 447
Levene, H., 385
Li, C. C., 535
Linsley, E. G., 15
Lizards, *Anolis*, thermal regulation, 98
Lycopersicon, seed dormancy, 407
Maguire, M. P., 394
Mallah, G. S., 1
Mammals of Australia, historical zoogeography, 431
Marklund, G., 510
Marler, P. (Book review), 382
Mather, W. B., 461
Mating propensity, estimation, 543
Mattoni, R. H. T., 447
Mayfield, H., 174
McNaughton, I. H., 209
Miller, W. J., 30; 196
Mimetic assemblages, lycid beetles, 15
Mimic model conditions, 85
Mimicry: Batesian, in snakes, 85; in lycid beetles, 15; Müllerian, evolution of, 567; Müllerian, in snakes, 85; in snakes, *Dasypeltis*, 72
Moore, R. J., 272
Morishima, H., 326
Moth, Food-plant specialization, 478
Natural selection between two species of fruit fly *Dacus*, 360
Natural selection, influence on hybrid *Helianthus*, 67
Neoteny in European lampreys, 530
Oka, H-I., 326; 418
Oryza perennis, differentiation in, 326; hybrids between *O. sativa*, 418
Oryza sativa, differentiation in, 326; hybrids between *O. perennis*, 418
Panaxia dominula, food-plant specialization, 478
Parasitism of Kirtland's warbler by cowbirds, 174
Parker, B. C., 228
Paratoid gland secretory substances in *Bufo*, 352
Pattern resemblance, in snakes, 86
Peromyscus truei species group, social discrimination, 555
Photoperiodism, phylogeny, polyploidy in Old World *Buddleia*, 272
Phyllocola petiti, possible phylogeny, 564
Phylogeny, polyploidy, photoperiodism in Old World *Buddleia*, 272
Pigeons, cellular antigens, 30
Pijl, L. van der, 44
Pittendrigh, C. S., chrmn., Biological clocks: Cold Spring Harbor Symposia Quant. Biol. (P. Marler), 382
Plantago lanceolata, leaf length responses of gamodemes, 166
Plethodon, color variant, 281
Poeciliopsis, reproductive mechanism of unisexual and bisexual strains, 302
Pollen vectors, gallwasp (*Blastophaga*) of fig tree (*Ficus*), 378
Pollination of zoophilous flowers, 44
Polyploidy, phylogeny, photoperiodism in Old World *Buddleia*, 272
Population dynamics of a variant *t*-allele in house mice, 385
Populations, closely related species, 209
Populations of *Drosophila ananassae*, gene arrangements, 486
Populations of *Drosophila melanogaster*: heterosis, fitness, 496
Populations, euryokous, in beetles, 2
Populations, experimental, of *Drosophila persimilis*, 340
Populations, geographic, of *Drosophila pavani*, 92
Populations, *Helianthus*, 60
Populations, *Rana*, histocompatibility in, 267
Populations, stenokous, in beetles, 2
Populations, Wild Rice, directions of differentiation, 326
Predation on lycid beetles, 24

Pseudogamy and evolution in *Ranunculus* group, 510
Ranunculus auricomus, evolution, 510
Rattus, genetic drift, 259
Rees, H., 145
Rensch, B., Evolution above the Species Level (G. G. Simpson), 112
Reproduction, mechanism in unisexual and bisexual *Poeciliopsis*, 302
Reproductive isolation, effect and fate in *Helianthus* populations, 67
Rick, C. M., 407
Rousi, A., 510
Ruibal, R., 98
Sagar, G. R., 209
Salamander, color variant, 281
Schultz, R. J., 302
Secretions of paratoid glands in *Bufo*, 352
Seed dormancy in Galápagos tomatoes, 407
Simpson, G. G. (Book review), 112; Principles of Animal Taxonomy (Th. Dobzhansky), 263; 431
Skin homografts in bullfrog populations, 267
Snakes, mimicry in *Dasypeltis*, 72
Social discrimination between *Peromyscus comanche, nasutus, truei*, 555
Soil algae, ecological niche relationships, 228
Species closely related, evolution and ecology, 209
Spiess, E. B., 340; 535
Springtails, cave forms, convergence and parallelism, 288
Stebbins, G. L., 60
Stormont, C., 196
Sun-flowers, changes in variation pattern, 60; morphological and cytological variation, 247
Suzuki, Y., 196
Syndrome, co-adaptive, in zoophilous flowers, 50
Tamsitt, J. R., 555
Tantawy, A. O., 1; 132
Temperature effects in *Drosophila*, 1
Termites, vestigial characters; regressive evolution, 115
Tetrapod origin and larval competition, 566
Thurow, G. R., 281
Tinderholt, V. E., 447
Tripsacum and *Zea* chromosome divergence, 394
Turner, B. L., 228; 352
Uhl, C. H., 375
Variation, geographical, *Drosophila*, 1
Variation in hybrid *Helianthus population*, 60
Variation, intraspecific in wing length and body size in birds, 180
Variation, morphological, cytological in *Helianthus petiolaris*, 247
Vecht, J. van der, 468
Warburton, F. E., 566
Wasps, Indo-Australian, evolution of, 468
Watson, P. J., 166
Wild rice, directions of differentiation in populations, 326
Zanandrea, G. S. J., 523
Zea and *Tripsacum* chromosome divergence, 394
Zoogeography, historical, of Australian mammals, 431

INFORMATION FOR CONTRIBUTORS

Contributions to EVOLUTION may be in the fields of genetics, cytology, plant and animal taxonomy, paleontology, ecology, anthropology, bacteriology, or any other field of biology, provided that the facts and conclusions presented are of primary interest to the student of evolution. Descriptive material shall be included only so far as it is the basis of the evolutionary conclusions. Manuscripts are examined for suitability by members of the Editorial Board or other competent critics.

The length of manuscripts will be limited to twenty printed pages unless an exception is granted by the Editorial Board. Short manuscripts and discussions will be included in a separate section, "Notes and Comments." Half tones and tabular material will have to be kept within economic limits.

Proof should be corrected immediately on receipt, and returned at once with manuscript to the Managing Editor. Authors should leave forwarding directions whenever they move away from the address sent with the manuscript. Ordinarily page proof will not be sent to the author. Costs of changes in proof from the original copy will be charged to the author.

Reprints should be ordered when galley proof is returned. There is usually a delay of several weeks after publication before the author receives the reprints ordered. Illustrations and cuts will be destroyed, unless their return is requested on the reprint order.

Manuscripts should be typewritten in double spacing with ample margins and on only one side of the paper. The Editor should have *one carbon copy in addition* to the original manuscript. The author should keep one carbon copy.

Tables and captions for figures should be typed on separate sheets from the text. The legends for several figures may be placed on a single sheet.

Illustrations, including maps, should be referred to as "figures" wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures. All figures and plates are reduced to a maximum of 4⅜ inches in width and 6⅛ inches in length. Reductions may be stated in the caption but not on the figure.

All references to literature should be collected into a list of "Literature Cited" at the end of the article. See recent numbers of EVOLUTION for accepted style.

Titles should be short, preferably less than seven words. Footnote material should be incorporated in the text whenever possible.

Every article of more than a few pages should have a summary limited to less than four per cent of the length of the paper; it is to be complete in itself, and understandable without reference to the body of the article. The scope of the paper should be stated in an introductory paragraph.

Authors are requested to prepare abstracts of their papers to be returned with the proof to the Managing Editor to be forwarded to Biological Abstracts.

Manuscripts should be addressed to

Charles D. Michener
Department of Entomology
University of Kansas
Lawrence, Kansas

THE SOCIETY FOR THE STUDY OF EVOLUTION

Officers for 1961

President: F. HARLAN LEWIS, University of California, Los Angeles, Calif.

President-elect: W. FRANK BLAIR, University of Texas, Austin, Tex.

Vice-Presidents: JOHN A. MOORE, Columbia University, New York, N. Y.
EVERETT C. OLSON, University of Chicago, Chicago, Ill.
M. J. D. WHITE, University of Melbourne, Melbourne, Australia

Secretary (1959–1961): HERBERT H. ROSS, State Natural History Survey, Urbana, Ill.

Treasurer (1961–1963): ROBERT E. KEER, University of Kansas, Lawrence, Kansas.

Editor (1959–1961): I. MICHAEL LERNER, University of California, Berkeley, Calif.

Managing Editor (1961–1963): D. DWIGHT DAVIS, Chicago Natural History Museum, Chicago 5, Ill.

Past Presidents: A. E. EMERSON (Chicago); E. ANDERSON (St. Louis)

Elected Members of the Council:

Class of 1961: W. FRANK BLAIR (Austin); JENS CLAUSEN (Stanford)
Class of 1962: ANTONIO B. DA CUNHA (São Paulo); CHARLES D. MICHENER (Lawrence)
Class of 1963: DEAN AMADON (New York); VERNE GRANT (Claremont)

OBJECTS: The object of the Society for the Study of Evolution, which was founded in March, 1946, is the promotion of the study of organic evolution and the integration of the various fields of science concerned with evolution. The Society endeavors to accomplish this through the publication of the journal and through meetings and working committees.

MEMBERSHIP: Membership is open to all those who are interested in the study of evolution. Members are elected by the Council. There are three classes of members:

Members—Dues $7.00, including subscription to EVOLUTION
Sustaining Members—Dues $14.00
Patrons—Single fee $1,000.

All members in good standing receive EVOLUTION.

Applications for membership should be directed to the Secretary, to whom all correspondence regarding membership should be addressed. Institutions may not become members but may subscribe to EVOLUTION. All checks should be made payable to The Society for the Study of Evolution.